

CARDIOVASCULAR AND RESPIRATORY
EFFECTS OF
THREE FISH ANAESTHETICS

A thesis submitted in partial fulfilment
of the requirements for the Degree of
Doctor of Philosophy in Zoology
at the
University of Canterbury,
Christchurch, New Zealand

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1999

To my family,
Mum, Dad and Stephen.

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Abstract

Whole animal and *in vitro* experiments were used to compare and contrast the cardiovascular and, to a lesser extent, respiratory effects of three fish anaesthetics at approximately equipotent concentrations: 100 ppm MS222, six to ten ppm metomidate and 60 ppm Aqui-S. Heart rate, cardiac output, dorsal aortic pressure, stroke volume, haematocrit, PaO₂ and plasma catecholamines were measured during five minutes of anaesthetic exposure with no other obvious stressors. Observed changes were due to both direct anaesthetic effects and secondary consequences of anaesthesia. Cardiovascular changes and heightened activity of Aqui-S treated fish suggests this anaesthetic causes a nociceptor mediated response.

In vitro investigations of branchial haemodynamics, using isolated perfused gill preparations, vasoactivity of branchial arteries, using myography, changes in myocardial contractility of the ventricle, using *in vitro* paced ventricle strips, and changes in the ability of the stimulated vagus nerve to reduce heart rate, using a spontaneously beating *in situ* heart and nerve preparation, were performed to help explain the cardiovascular changes in the whole animals during anaesthetic induction. Using the results from these studies and information in the literature the responses of the whole animal were divided into four groups: primary effects were those caused directly by the anaesthetic, secondary effects were caused as a consequence of the primary effects, tertiary effects were due to a reaction to the presence of the anaesthetics and human effects were caused by handling or other manipulation of fish. The cardiovascular effects of anaesthetic induction after handling were quite different from those during unstressed anaesthetic induction, and were mostly anaesthetic independent which suggested that the effects of handling were greater than the effects of anaesthetic exposure.

Oxygen consumption during 24 hours recovery from anaesthesia showed there were no long term changes in respiration. Measurements of heart rate, cardiac output, dorsal aortic pressure and stroke volume during six hours recovery from five minutes exposure to the three anaesthetics showed recovery of these variables occurred within 30 minutes. However, Aqui-S treated fish showed an elevated stroke volume 30 minutes into the recovery. Measurements of heart rate, cardiac output and stroke volume during recovery from anaesthesia and surgery suggested the cardiovascular effects were at least partially dependent on the anaesthetic used. However, the difference between the cardiovascular effects with and without surgery were far greater than any between anaesthetic effects.

Chapter 1

Introduction

PREFACE

Literature concerning the physiological effects of fish anaesthesia often focuses on haematological analyses and the measurements of stress. This is understandable as fish anaesthesia is generally employed as a tool to temporarily increase the manageability of fish, where paralysis or sedation and post-anaesthesia survival and health (which can be directly attributable to stress) are the main requirements. Information on cardiovascular effects is limited to about ten publications. Research focussing on the cardiovascular system has been performed previously using MS222 (a.k.a. tricaine, Finquel™, metacaine, ethyl *m*-aminobenzoate methanesulphonate) in brook trout (*Salvelinus fontinalis*) and, to a lesser extent, in carp (*Carassius carassius*) (Houston *et al.*, 1973; Houston *et al.*, 1969; Houston *et al.*, 1971a; Houston *et al.*, 1971b; Houston and Woods, 1972). These investigations were extensive but few cardiovascular variables were measured and the studies only examined one anaesthetic. Fredricks *et al.* (1993) looked in detail at the cardiovascular effects of MS222, benzocaine, 2-phenoxyethanol and etomidate during induction and recovery on spinally transected rainbow trout (*Oncorhynchus mykiss*) and measured both dorsal and ventral aortic blood pressures and recorded their electrocardiograms (ECG). However, the experiments could only be performed under very controlled conditions, as the fish were paralysed posterior to the head in an attempt to reduce noise in the ECG recordings, and therefore were unlikely to represent the typical situation during commercial use of these fish anaesthetics. There are few other studies looking at the effects of anaesthesia on the cardiovascular system of fishes (Campbell and Davies, 1963; Lochowitz *et al.*, 1974; Randall, 1962; Ryan *et al.*, 1993; Serfaty *et al.*, 1959; Yamamitsu and Itazawa, 1988). This is surprising considering that the cardiovascular effects of anaesthetics on humans and other mammals are well documented in textbooks (Aitkenhead and Smith, 1996; Brander *et al.*, 1982; Gray *et al.*, 1979; Hall and Clarke, 1991; McDonald and Wann, 1978; Stoelting and Miller, 1989).

Before discussing the physiological effects of fish anaesthesia an understanding of the fundamental aspects of fish anaesthesia and, where relevant, vertebrate anaesthesia is desirable. Most of the remainder of this chapter is dedicated to this end. The final sections describe the aims of this study and introduce the anaesthetics examined.

Table 1.1. Types of fish anaesthesia available.

Group	Subgroup	Notes
Chemical	inhalation	can use a dissolved solid, gas or liquid
	injection	can be injected into a number of blood spaces, vessels, muscles or cavities
	drugged food	eg. (Murai <i>et al.</i> , 1979) drugged fish food with valium
Physical	electrical	AC or DC
	cold	eg. using an ice slurry
	stunning	not easily controlled and not very humane

METHODS OF FISH ANAESTHESIA

This work focuses on chemical inhalation anaesthesia using a dissolved solid or liquid anaesthetic. This is the most common type of fish anaesthesia but there are a number of others. Table 1.1 summarises the different types of fish anaesthesia available.

USING CHEMICAL INHALATION FISH ANAESTHETICS

Generally fish are added to a container of anaesthetic solution of the correct concentration, or the correct amount of raw anaesthetic or volume of concentrated anaesthetic is added to the water in which the fish are swimming. For prolonged anaesthesia a recirculating anaesthetic solution can be used where oxygenated anaesthetic is passed over the gills, collected and reoxygenated for another pass over the gills (Brown, 1986). A novel idea for large elasmobranchs and large trout was to spray the gills with a concentrated anaesthetic solution (Gilbert and Wood, 1957; Kidd and Banks, 1990). This was stated to have worked quickly and effectively.

USERS AND USES OF FISH ANAESTHETICS

The aquaculture industry is the largest user of fish anaesthetics by volume. The tasks anaesthetics facilitate include taking of growth measurements, harvesting, spawn taking, transportation, tagging and vaccinating. With these tasks the objective of anaesthetization is to increase the manageability of fish. Researchers are probably the second largest users but the quantities used would be much smaller than those used by the aquaculture industry. Researchers use anaesthetics for a variety of tasks such as preparing fish for invasive procedures, field collection from tide pools and using

scuba, and examining dangerous or fragile species. For some of the tasks general anaesthesia is necessary whereas for others only sedation and/or immobilisation are required. Other professionals that make use of fish anaesthetics are veterinarians and photographers.

A BRIEF HISTORY OF FISH ANAESTHETICS

Early methods of fish anaesthesia include “a blow to the head from which, with luck, the animal might later recover,” electrical stunning or “a few spoonfuls of brandy or whiskey added to the water” (Healey, 1964). Later, general anaesthetics such as ether and chloroform were tried, as well as a number of other chemicals such as urethane, menthol, chloretone, chloral hydrate and cocaine. Many of these have since been replaced. In the early 1940s urethane was introduced as a fish anaesthetic and was commonly used by physiologists and aquaculturists in the latter part of the decade. In 1956 its carcinogenic nature was discovered and its use abandoned (Ball and Cowen, 1959; Summerfelt and Smith, 1990). Similarly, other chemical anaesthetics have lost favour due to their carcinogenic nature or toxicity (eg. chloroform is carcinogenic, chloretone is not used in Norway due to its toxicity, Malmstrom *et al.*, 1993). Ether was also quite popular but its use waned due to its flammable nature and low flash point. Chilling using crushed ice has been used (Parker, 1939) but it causes stress and the anaesthesia achieved is not very deep (Healey, 1964; McFarland, 1960). More recently it has been used as a supplement to chemical anaesthesia (Williamson and Roberts, 1981).

MS222 became popular in the 1960s and has remained popular probably because it is the only fish anaesthetic registered by the American Food and Drug Administration (FDA). Fish anaesthetised with MS222 must have a 21 day withholding period before being sold for food. Other effective and cheaper anaesthetic agents are available (eg. quinaldine, benzocaine, 2-phenoxyethanol) but cannot be used on food fish. Carbon dioxide anaesthesia, using CO₂ gas or by reacting sodium bicarbonate with acid (Fish, 1943), is also used as it is Generally Regarded as Safe (GRAS) by the FDA and can be used on food fish without restrictions. Many other chemicals have been tried but their use has not been widely established (eg. etomidate, metomidate, propanidid, many barbiturates, clove oil and propoxate).

PROPERTIES OF AN IDEAL FISH ANAESTHETIC

A list of qualities for an ideal anaesthetic has been proposed (Bell, 1987). These are summarised in Table 1.2. As aquaculturists are the largest anaesthetic users, anaesthetics tend to be judged

Table 1.2. Qualities of an ideal fish anaesthetic. Abbreviated from Bell (1987).

Factor	Comment
inexpensive	per effective dose
non-toxic/irritant	to fish and user
rapid acting	immobilise quickly without causing stress
solubility	readily water soluble, maximum solubility lower than lethal dose
stability	stable stored and in solution, no reaction with light, heat, seawater
rapid recovery	to prevent mortalities
body clearance	useful to fish and consumer
biodegradable	for easy and safe disposal
non-foaming	foam may interfere with observation and air/water gas exchange

on their ability to fulfill the needs of this group. The information in Table 1.2 reflects this perspective. Most of the factors mentioned in Table 1.2 are of value to other users as well but some important factors are missing. Researchers would prefer an anaesthetic to have a minimal effect on both physiology (McFarland and Klontz, 1969) and behaviour, as experiments on fish in a state close to normality will give more reliable results. Aquaculturists' and researchers' objectives are becoming more alike as the increasing knowledge of aquaculture methods suggests unstressed fish grow more quickly and produce a superior product (Jerrett *et al.*, 1996; Ross and Ross, 1984; Sumpter *et al.*, 1993).

DEFINITIONS OF ANAESTHESIA

Anaesthesia is a non-specific word used to describe a broad range of states. Its simplest definition is "an absence of sensation" or "an artificially induced inability to feel pain" (Brown, 1993). Unfortunately, this is very similar to the definition for analgesia which is an "absence or reduction of ability to feel pain" (Brown, 1993). Anaesthesia and general anaesthesia tend to be thought of as synonymous although there are (local) anaesthetics, which do not cause general anaesthesia, and a number of other types of drugs that have some of the same or similar properties as general anaesthetics. Hypnotics induce sleep but have no analgesic or muscle relaxing properties. Narcotics, such as chemicals from the morphine group, induce a stuporous condition or sleep and may have analgesic properties. Muscle relaxants paralyse muscles by blocking neuromuscular channels. Finally, local anaesthetics cause analgesia and paralysis by blocking nerve conduction. Local anaesthetics act locally, on the nerves and muscle at the site of application, whereas general anaesthetics act on the central nervous system.

Table 1.3. Stages of anaesthesia as proposed by McFarland (1959).

Stage	Plane	visual stimulus (shadow over jar)	vibrational stimulus (tap jar rim)	equilibrium (Eq.) muscle tone (MT)	Respiration rate
0		Reactive	Reactive	Normal	Normal
1	1	Slightly reactive	Reactive	Normal	Normal
	2	No reaction	Slight to no reaction	Normal	Normal
2	1	No reaction	Slight to no reaction	Partial loss Eq	Increased rate
	2	No reaction	Slight to no reaction	Complete loss of Eq and MT	Rapid decline
3		No reaction	No reaction	Complete loss of Eq and MT	Slow amplitude decrease
4		No reaction	No reaction	Complete loss of Eq and MT	Ceases

STAGES OF FISH ANAESTHESIA

McFarland (1959) produced one of the first complete “stages of anaesthesia” charts for fish (Table 1.3). This was based on information of equivalent human stages of anaesthesia because of “the striking parallel of anaesthesia in fishes and higher vertebrates”. The concentrations of anaesthetics he used were low compared to those used commonly today and the observation periods were up to 12 hours. At higher anaesthetic concentrations some of the stages that McFarland (1959) proposed occur too quickly to be observed (Ross and Ross, 1984). There have been many “improved” or “simplified” versions of McFarland’s chart (Bell, 1987; Brown, 1988; Iwama *et al.*, 1989; Ryan, 1992a; Ryan, 1992b; Schoettger and Julin, 1967) but the fundamental structure remains the same. There is progressive loss of reactivity to stimuli, loss of motor skills and reduction in ventilation frequency and amplitude. The decline in ventilation eventually leads to death from hypoxia if the concentration of anaesthetic is sufficiently high.

TYPES OF ANAESTHETICS

There are two basic types of anaesthetics: general anaesthetics, which primarily affect the central nervous system, and local anaesthetics, which cause analgesia at a specific site. These will be discussed in the next two sections. Hypnotics are briefly covered in the general anaesthetic section. In the third section fish anaesthetics will be discussed, not because they are different from the other two types of anaesthetics, but because fish anaesthetics comprise an unresolvable mixture of local anaesthetics, general anaesthetics, hypnotics and other chemicals.

GENERAL ANAESTHESIA

The information in this section mostly pertains to, and was gained from studies upon, humans and other mammals. Anaesthesia is a state in which sensations are not perceived. General anaesthesia produces this state via unconsciousness (Brander *et al.*, 1982). Other definitions of general anaesthesia tend to be more specific but differ between authors. Brown (1988) uses the mammalian definition: that general anaesthesia offers three separate effects; analgesia (the inability to feel pain), narcosis (unconsciousness) and skeletal muscle relaxation (Brown, 1988). During general anaesthesia skeletal muscle relaxation is caused by central inhibition. Normally there is a slight tension in muscle (tone or tonus) due to impulses from cells in the ventral horn region of the spinal cord. During anaesthesia central nervous system activity decreases which in turn decreases ventral horn activity causing muscle relaxation (Hall and Clarke, 1991). Muscle can also relax via the direct effect of some anaesthetics on muscle fibres. Some authors are specifically interested in anaesthesia as a tool for surgery. For example, it has been suggested that general anaesthesia is a "state of unconsciousness produced by a process of controlled, reversible drug induced intoxication of the central nervous system in which the patient neither perceives nor recalls noxious stimuli" and that any other effects are "alternative pharmacological processes" (Hall and Clarke, 1991). Hall and Clarke also state that hypnosis is not a part of anaesthesia and that anaesthesia is an "all or nothing" state with no stages. This may be a useful definition for general anaesthesia as required for surgery but it may be too restrictive for other purposes.

The site of action of general anaesthetics is the central nervous system. The central nervous system controls what is felt or not felt (Richards, 1980). It has been suggested that, in humans, areas of the brain are affected sequentially from the highest (cerebral cortex) to the lowest (spinal cord) and that fish are affected similarly (McFarland, 1959). McFarland (1959) stated that:

".... the striking parallel of anaesthesia in fishes and higher vertebrates, as well as a basic morphological similarity of the nervous systems, leaves the impression that fundamental processes in anesthesia are produced through a suppression of neural centres, which, if not morphologically homologous, are at least in part, functionally equivalent".

Evidence for this statement came from studies using brain ablation and from sequence of anaesthesia charts for humans. This idea is also supported by the observation that anaesthetics do not cause a uniform change in the activity of the brain (Richards, 1980). Other authors do not believe that areas of the brain are sequentially depressed (Hall and Clarke, 1991).

The mechanism of action of general anaesthetics is still unknown. It is generally accepted that general anaesthetics act at membranes (Richards, 1980). What they act on and what is ultimately

the effector of general anaesthesia is the subject of continued research. The fact that some anaesthetics have synergistic effects suggests that not all anaesthetics work at the same sites (Richards, 1980).

Originally it was proposed that anaesthetics disrupt the cell membrane causing leaking of ions. This hypothesis is now in disfavour as the anaesthetic concentration required to cause membrane disruption is higher than that needed to cause anaesthesia (Franks and Lieb, 1982). Another idea was that some anaesthetics alter the membrane matrix around vital membrane proteins which alters the proteins' function. This is supported by the fact that many anaesthetics are chemically inert (eg. xenon, alkanes) and that there is a good correlation between an anaesthetic's potency and its olive oil solubility (McDonald and Wann, 1978; Richards, 1980). The correlation is stronger if octanol is used instead of olive oil, and stronger still using lipid bilayers (Janoff *et al.*, 1981). High pressure can negate the effects of gaseous anaesthetics. This could suggest that anaesthetics increase the membrane volume at the site (eg. cytoplasmic space between lipid bilayer) and that increased pressure squeezes out the anaesthetic (Franks and Lieb, 1982) or presses the site into its original shape.

Other anaesthetics may modify the functions of membrane proteins via direct, reversible, non-covalent binding to specific membrane protein sites (Richards, 1980) or by competing with ligands for active sites on ligand gated channels (eg. GABA_A, glycine) (Franks and Lieb, 1994). The supporting evidence is that smaller molecules in a family (eg. the alkanes) act as anaesthetics whereas larger molecules (eg. decane) of the same family do not, which suggests molecules can become too large for a site. It could also suggest that the chemical nature of the molecule has altered enough so that the chemical can no longer get to the site by being, for example, too hydrophobic (Franks and Lieb, 1982). Some anaesthetics may have a general effect whereas others may be very selective about the types of proteins, protein subunits or even subunit isoforms on which they act (Duch *et al.*, 1995; Tonner and Miller, 1995). Further possible mechanisms are discussed by Franks and Lieb (1982, 1994).

There have been a number of side effects observed during general anaesthesia in mammals, some of which are generalised and others specific to certain drugs. General anaesthetics can affect the cardiovascular system. Possible changes include a (dose dependent) decrease in arterial blood pressure and cardiac output due to a decrease in myocardial contractility (possibly caused by impaired calcium movement in muscle tissue, which may also reduce stroke volume) and/or a decrease in systemic resistance caused by peripheral vasodilation (Hall and Clarke, 1991; Stoelting and Miller, 1989). Heart rate may increase as a reflex to a decrease in blood pressure (via carotid baroreceptors) unless the baroreceptors or medullary vasomotor and respiratory centres of the

brain are impaired, which would reduce the ability of the cardiovascular system to react to changes in blood volume and blood pressure (Hall and Clarke, 1991). This is not always the case as *in vitro* halothane, enflurane, isoflurane and nitrous oxide decrease myocardial activity in a dose dependent manner whereas *in vivo* this is not observed. The absence of an effect *in vivo* is probably due to compensatory homeostatic mechanisms, especially autonomic nervous system activity (Stoelting and Miller, 1989). In addition, there may be a decrease in the amount of catecholamines released from the adrenal medulla, possibly caused by a decrease in transmission of autonomic impulses through the autonomic ganglia, which could further impair homeostatic mechanisms designed to increase blood flow and maintain blood pressure. These changes are a mixture of direct effects and reflex changes and are often drug dependent.

All anaesthetics cause a dose dependent decrease in muscle tone causing a decrease in depth and minute volume of breathing which, in the case of an overdose, can lead to complete respiratory muscle paralysis (Hall and Clarke, 1991). If the depth of ventilation decreases, rate may increase as a reflex (Hall and Clarke, 1991). Different animals have their sensitivity to O₂ and CO₂ levels depressed which reduces their drive to breathe leading to irregular breathing or apnoea (Hall and Clarke, 1991). Some drugs cause a dose dependent and drug specific depression of ventilation due to a direct effect on both the medullary centre and on the intercostal muscles (Stoelting and Miller, 1989). An increase in ventilation rate via central nervous system stimulation may also occur, but the decrease in tidal volume is greater causing an overall decrease in minute volume (Stoelting and Miller, 1989).

Hypnotics, such as metomidate, etomidate, propofol, urethane, chloral hydrate and trichloroethanol (McDonald and Wann, 1978), are usually grouped in with the general anaesthetics. These drugs induce a sleep like state and have been used prior to a general anaesthetic or analgesic before surgery.

LOCAL ANAESTHETICS

Local anaesthetics produce analgesia and muscle relaxation by blocking axonal impulse conduction in nerves (Rowland, 1974) and by interfering with ion movements in muscle (McDonald and Wann, 1978). Cocaine was the first local anaesthetic used and was introduced to medicine in 1884. Cocaine had some irritant properties and could cause psychological dependence so a search for a synthetic substitute was initiated. The first synthetic drug was introduced in 1905 (Stoelting and Miller, 1989) and since this time numerous drugs have been tried and tested as local anaesthetics.

Local anaesthetics are relatively small, organic molecules possessing a lipophilic portion (usually an unsaturated benzene ring), a hydrophilic portion (a tertiary amine) and either an ester or an amide as a carbon link. The ester and amide drugs have different effects and sites of metabolism. Local anaesthetics have charged and uncharged forms of which the uncharged forms are lipophilic (Rowland, 1974).

To understand how local anaesthetics work a basic understanding of nerve conduction is required. A current is produced when there is a movement of charged particles. In nerves the movement is through channels across the membrane of the nerve axon and the moving charged particles are sodium and potassium ions. In a steady state, pumps in the axon membrane transport potassium ions into the cell in exchange for sodium ions. This produces a potential across the membrane called the resting potential. When a nerve is stimulated, channels in the membrane nearest the point of stimulation open allowing sodium ions to move into the cell, down the concentration and potential gradient, which decreases the transmembrane potential (depolarisation). If this potential decrease is greater than the threshold potential then voltage gated channels around this site open allowing movement of more sodium ions. This produces a chain reaction in the axon membrane producing a continuous action potential down the length of the nerve (Hill and Wyse, 1989). Local anaesthetics produce a reversible conduction blockade of the nerve impulse (Stoelting and Miller, 1989) by inhibiting sodium and potassium ion flow through the channels in the axonal membrane (Århem and Frankenhaeuser, 1974). This slows the rate of change of the transmembrane potential (depolarisation rate) so that the threshold potential is reached more slowly or not reached at all, which either slows the action potential conduction velocity or stops the action potential completely. The refractory period, when the membrane is repolarising by reestablishing its resting potential, is also increased (Corvino, 1980). Local anaesthetics do not alter the resting potential (Corvino, 1980; Stoelting and Miller, 1989) but there is some disagreement over whether they increase (Levy, 1974) or do not affect (Corvino, 1980; Stoelting and Miller, 1989) the excitation threshold potential. Different nerve types have different properties which alter their sensitivities to local anaesthetics. For example, motor fibres are twice as resistant to local anaesthetics as sensory fibres (Stoelting and Miller, 1989).

Side effects from local anaesthetics generally occur when the chemical is allowed to enter the circulation in large enough quantities. Local anaesthetics affect all nerves and muscle types relatively non-specifically and, by entering the blood, local anaesthetics have access to many vital nerves and muscles. Blood borne local anaesthetics can affect the central nervous system, cardiovascular system, and respiration (Hall and Clarke, 1991). Also, at the intended site of action these drugs can be toxic in high concentrations or cause an allergic reaction (Stoelting and Miller, 1989).

The effect on the human central nervous system can range from mild sedation, restlessness, vertigo and slurred speech to *grand mal* clonic seizures (Hall and Clarke, 1991; Stoelting and Miller, 1989). It is thought that this is due to the local anaesthetics initially blocking inhibitory cells which can lead to excitation and convulsions. With higher concentrations excitatory cells are also affected causing a cessation of central nervous system activity leading to apnoea and death (Hall and Clarke, 1991; Stoelting and Miller, 1989). Hypercapnia tends to potentiate local anaesthetic effects on the brain (Hall and Clarke, 1991).

Local anaesthetics can affect the intrinsic rhythm of the heart and its conduction pathways. At low doses local anaesthetics (eg. lignocaine) can act as anti-arrhythmics, by decreasing the action potential and refractory period, and at higher concentrations have been known to cause sinus bradycardia which can lead to cardiac arrest (Hall and Clarke, 1991). At high concentrations local anaesthetics can have negative inotropic effects. Local anaesthetics are known to decrease the contractility of the mammalian myocardium decreasing cardiac output (Hall and Clarke, 1991). *In vitro* fish hearts are similarly affected by MS222 (Ryan *et al.*, 1993). All local anaesthetics cause vasoconstriction at low concentrations and vasodilation at high concentrations (Hall and Clarke, 1991). It is thought that local anaesthetics in the charged form block contraction, and in the uncharged form cause calcium release from the sarcoplasmic reticulum producing muscle contraction (McDonald and Wann, 1978). Systemic hypotension can occur due to sympathetic blockade during epidural block (Hall and Clarke, 1991) and also due to arteriolar vascular smooth muscle dilation and direct myocardial depression. Renal and hepatic blood flow can be attenuated due to central nervous system effects which can decrease the rate of excretion and metabolism of anaesthetics prolonging other effects (Hall and Clarke, 1991).

At the lungs bronchial smooth muscle relaxes and ventilation rate can decrease due to central nervous system effects (Hall and Clarke, 1991). Similarly, it may be expected that in the gills of fishes relaxation of muscle fibres in the blood vessels and pillar cells could occur, possibly leading to changes in gill circulation and haemodynamics. At the site of action, large doses of anaesthetic can cause damage to nerve and muscle cells. An allergic reaction can occur in some subjects (Hall and Clarke, 1991; Stoelting and Miller, 1989).

Many fish anaesthetics were designed or have been used as local anaesthetics (eg. MS222, benzocaine, lidocaine, clove oil). Surprisingly, given the many deleterious side effects seen when local anaesthetics enter the blood, the aim of anaesthetizing fish with these is to get the drug into the blood. These chemicals probably cause general anaesthesia in fish which shows that they are classified by how they are used rather than by their actual properties.

FISH ANAESTHETICS

It is generally assumed that fish anaesthesia is general anaesthesia even though local anaesthetics and hypnotics are sometimes used. The primary site of action of general anaesthetics is the central nervous system so a correlation between depth of fish anaesthesia and the brain concentration of anaesthetic may be expected. A number of workers have looked for a correlation between MS222 concentration in certain tissues and depth of anaesthesia. Their conclusions have varied with correlations being found between depth of anaesthesia and MS222 concentration in brain tissue (Hunn, 1970; Ryan, 1992b), blood (Houston and Woods, 1976; Ryan, 1992b; Stenger and Maren, 1974) and interstitial fluids (Houston *et al.*, 1976; Houston and Woods, 1976). In one case no correlation was found (Ryan, 1992a) even though MS222 concentrations were measured in the brain, blood, liver, kidney and white muscle. MS222 may not be a suitable fish anaesthetic to look for this correlation because it also has local anaesthetic properties and may work on sites other than in the central nervous system. It has been reported that an isobutyl homologue of MS222, eight times more potent than MS222, injected intravenously or intramuscularly causes anaesthesia in the dogfish (*Squalus acanthias*) without the drug reaching the brain or appearing in the cerebrospinal fluid (Stenger and Maren, 1974). These authors also found no correlation between depth of anaesthesia and brain or cerebrospinal fluid MS222 concentrations. They concluded that MS222 probably causes neuromuscular blockade, like a muscle relaxant, rather than acting like a general anaesthetic. However, their experiments showed MS222 injected intravenously did not cause anaesthesia. Similarly, it has been shown that benzocaine injected intraperitoneally at concentrations up to 120 mg.kg⁻¹ does not cause anaesthesia in trout (*Salmo trutta* or *Oncorhynchus mykiss*) (Oswald, 1978). What the experiment of Stenger and Maren (1974) possibly shows is a part of the action of MS222, as MS222 also has analgesic properties and more importantly, as it is a local anaesthetic, it almost certainly causes central nervous system dysfunction. The loss of balance control, directional stability, and locomotory coordination in *Pagrus auratus* as sedation increased to anaesthesia all point to the disruption of brain function (Ryan, 1992b). However, the results of Stenger and Maren (1974) do emphasise the fact that the external appearance of general anaesthesia in fish is the same as the external appearance of paralysis or complete skeletal muscle relaxation and that care must be taken in assuming that an immobilised fish is not aware or that it cannot feel pain. Conversely, fish anaesthetised with metomidate or its analogues may still have skeletal muscle movement and yet be totally under the influence of the hypnotic.

ANAESTHETIC UPTAKE AND ABSORPTION

Fish inhalation anaesthetics are mainly taken up by the gills although some enters fish across the skin (Ferreira *et al.*, 1984a). The gills are ideal sites for transfer of small, lipophilic, neutral molecules as they receive virtually all the cardiac output, are composed of very thin layers of lipid rich cells and have a large surface area to volume ratio that favours material exchange with the environment (Meinertz *et al.*, 1991). The rate of uptake, and hence the induction time, should be proportional to the products of branchial surface area, ventilation rate, blood flow through the gills and anaesthetic concentration (Houston and Woods, 1976). The concentration of MS222 in the blood of rainbow trout (*Oncorhynchus mykiss*) reaches 50% of the water concentration of MS222 within two minutes (Houston and Woods, 1976), 100% in dogfish (*Squalus acanthias*) within two minutes (Stenger and Maren, 1974), and 79% in *Pagrus auratus* within two minutes (Ryan, 1992b). In rainbow trout, it was found that irrigating the gills with the anaesthetic solution increased blood MS222 concentration to greater than that of the anaesthetic solution (Houston and Woods, 1976).

Anaesthetic uptake and hence induction time are affected by a number of factors. These are summarised in Table 1.4. Not all of the findings agree with each other and the effect of some of the factors seems to be quite species dependent. For this reason the literature often suggests that, when working with a new species, always test the anaesthetic on a small number of fish first to work out correct anaesthetic doses and induction times.

ANAESTHETIC REMOVAL AND RECOVERY

Recovery from anaesthesia occurs when the anaesthetic stops interacting with the active sites. This can occur by way of removal or inactivation of the drug. Which of these has the greatest effect depends on the anaesthetic and the animal. In mammals, anaesthetic gases tend to be relatively inert and are generally expelled unaltered. In contrast, intravenous anaesthetics tend to be metabolised to an inactive form, which allows recovery, and are then excreted. Local anaesthetics are thought to be removed via vascular absorption, as they are injected into tissues and not into blood vessels, after which they are metabolised in other organs. Most fish anaesthetics are mammalian local or intravenous anaesthetics which suggests metabolism may be a major method of removal. However, evidence from studies on MS222 showed that recovery from, and metabolism of, MS222 was slower in lower vertebrates (amphibians and reptiles, metabolism half life of 8 - 112 minutes) than in higher vertebrates (mammals and birds, metabolism half life of 1.5 - 2.6 minutes) (Downes and Gerber, 1974; Wayson *et al.*, 1976). This difference may partially be caused by differences in temperature as the mammals and birds were 15 - 20 °C warmer than the

Table 1.4. Potential factors affecting anaesthetic uptake and induction.

Biological	species	<ul style="list-style-type: none"> - metabolic rate, size (Ross and Ross, 1984) - higher gill area/body mass ratio may decrease anaesthetic efficacy (Dixon and Milton, 1978) - inverse relationship between dose required and evolutionary status due to increased number of active sites in higher vertebrates (Ross and Ross, 1984) - different concentrations needed for different species (McFarland, 1959), (Späth and Schweickert, 1977) - MS222 induction of <i>Pagothenia borchgrevinki</i> much slower induction than in rainbow trout (<i>Oncorhynchus mykiss</i>) due to lower metabolic rate (Ryan, 1992a; Ryan, 1992b)
	size/weight	<ul style="list-style-type: none"> - size of salmonids had no effect on efficacy of MS222 (Schoettger and Julin, 1967) - efficacy greater on smaller fish eg. goldfish (<i>Carassius auratus</i>), brook trout (<i>Salvelinus fontinalis</i>) and rainbow trout (<i>Oncorhynchus mykiss</i>) (Houston et al., 1976); brown bullhead (<i>Ictalurus nebulosus</i>), Chinook salmon (<i>Oncorhynchus tshawytscha</i>) and rainbow trout (<i>O. mykiss</i>) (Gilderhus, 1989; Gilderhus et al., 1973); brook trout (<i>Salvelinus fontinalis</i>) (Houston and Woods, 1972); Porgy (<i>Pagrus major</i>) in MS222 (Oikawa et al., 1994); Atlantic salmon (<i>Salmo salar</i>) in metomidate (Olsen et al., 1995); goldfish (<i>Carassius auratus</i>) in 2-phenoxyethanol (Weyl et al., 1996); rainbow trout (<i>Oncorhynchus mykiss</i>) in 2-phenoxyethanol (Barton, 1981) - efficacy greater on larger fish eg. <i>Blennius pholis</i> in quinaldine (Dixon and Milton, 1978); brown bullhead (<i>Ictalurus nebulosus</i>) in benzocaine (Huish, 1972)
	age	<ul style="list-style-type: none"> - developmental stage (Ross and Ross, 1984) - older fish more oily, see lipid content (Ross and Ross, 1984)
	previous history	<ul style="list-style-type: none"> - injured, stressed, diseased, exhausted or post-spawn fish more susceptible (Ross and Ross, 1984) - 2-phenoxyethanol efficacy decreases with increasing number of exposures (Weyl et al., 1996) - some anaesthetic concentrations adequate initially were not adequate on successive occasions (Gilderhus and Marking, 1987)
	sexual maturity + sex-	gravid fish more oily, see lipid content (Ross and Ross, 1984)
	lipid content	<ul style="list-style-type: none"> - oily more susceptible (Ross and Ross, 1984)
	gill	<ul style="list-style-type: none"> - gill lipid content increases uptake rate (Houston and Woods, 1976) - uptake rate proportional to branchial area × ventilation rate × blood flow × [anaesthetic] (Houston and Woods, 1976)
	hyponatremia	<ul style="list-style-type: none"> - lack of Na increases local anaesthetic activity (Roweland, 1974)
	Environmental	
	temperature	<ul style="list-style-type: none"> - induction time decreased outside normal temperature of fish (Sylvester, 1975) - lower temperature increases efficacy eg. sockeye salmon (<i>Oncorhynchus nerka</i>) in 2-phenoxyethanol (Sehdev et al., 1963); zebra danio (<i>Danio rerio</i>), black tetra (<i>Gymnocorymbus ternetzi</i>), angelfish (<i>Pterophyllum scalare</i>) and southern platyfish (<i>Xiphophorus maculatus</i>) in etomidate (Amend et al., 1982); goldfish (<i>Carassius auratus</i>) in MS222 (Baudin, 1932b); goldfish in 2-phenoxyethanol (Weyl et al., 1996) - higher temperature increases efficacy eg. rainbow trout (<i>Oncorhynchus mykiss</i>) in MS222 (Houston and Woods, 1976); MS222 and metomidate (Brown, 1988); salmonids in benzocaine (Gilderhus, 1989); channel catfish (<i>Ictalurus punctatus</i>), golden shiners (<i>Notemigonus crysoleucas</i>) and bluegills (<i>Lepomis macrochirus</i>) in etomidate (Limsuwan et al., 1983a), (McFarland, 1959); brook trout (<i>Salvelinus fontinalis</i>) in MS222 (Meister and Ritzi, 1958); sábalo (<i>Prochilodus lineatus</i>) in benzocaine (Parma De Croux, 1990); <i>Sarotherodon spiluris</i>, <i>Clarias batrachus</i>, <i>S. mossambicus</i>, <i>Tilapia mariae</i>, <i>S. galilaea</i> and <i>S. niloticus</i> in benzocaine (Ross and Geddes, 1979)
Anaesthetic	water hardness	<ul style="list-style-type: none"> - calcium decreases the permeability of gills and decreases efficacy (McFarland, 1959)
	pH	<ul style="list-style-type: none"> - higher pH increases the permeability of gills decreasing the induction time (McFarland, 1959) - mullet (<i>Mugil cephalus</i>) induction time in MS222 decreases outside normal environmental pH of fish (Sylvester, 1975)
	PO ₂	<ul style="list-style-type: none"> - induction time lower in low PO₂ (Sylvester, 1975)
	lipid+water solubility	<ul style="list-style-type: none"> - affects available anaesthetic and therefore rate of uptake (Ferreira et al., 1984b; Hunn and Allen, 1974)
	acidity	<ul style="list-style-type: none"> - acidity affects respiration and cardiovascular variables, see Biological, gill (Ferreira et al., 1984a)
Anaesthetic	degree of ionisation	<ul style="list-style-type: none"> - at higher pH more MS222 is in the non-ionised, active form (McFarland, 1959; Ohr, 1976) - skin of fish take up unionised benzocaine HCl as it is more lipid soluble (Ferreira et al., 1984a)
	concentration	<ul style="list-style-type: none"> - increased anaesthetic concentration decreases induction time (Amend et al., 1982; Huish, 1972; Meister and Ritzi, 1958) and increases depth of anaesthesia (Huish, 1972)

amphibians and reptiles and metabolic rate would consequently be higher. The half life of MS222 in fish is variable (20 minutes in rainbow trout (*Oncorhynchus mykiss*) (Houston and Woods, 1972), about six minutes in channel catfish (*Ictalurus punctatus*) (Hunn and Allen, 1974) and about 56 minutes in dogfish (*Squalus acanthias*) (Stenger and Maren, 1974)). However, the proportion excreted by the gill is generally much higher than from other organs (Hunn *et al.*, 1968; Maren *et al.*, 1968b) and that which is excreted across the gills is mostly unmetabolized (Stenger and Maren, 1974). The plasma half life of MS222 would be much higher if excretion of unmetabolised anaesthetic by the gill did not occur (plasma half life of MS222 for dogfish calculated to be 60 hours, Maren *et al.*, 1968a). MS222 excreted in the urine (15 - 21% in rainbow trout) has mostly been metabolised to non-polar forms (Hunn *et al.*, 1968; Maren *et al.*, 1968a). Little work of this nature has been performed on other fish anaesthetics. A study of benzocaine in rainbow trout (*Oncorhynchus mykiss*) showed that, of the 70% of drug recovered, 59% was excreted by the gill, 9% in the urine and 2% in bile (Meinertz *et al.*, 1991). The half life of 2-phenoxyethanol in rainbow trout was calculated to be about 30 minutes (Imamura-Kojima *et al.*, 1987). A comparison of etomidate recovery in humans, on which it is used as a short acting hypnotic (Reneman and Janssen, 1977), and fish, where it is said to have a long recovery period (Amend *et al.*, 1982; Gilderhus and Marking, 1987) suggests that the difference in the rates of metabolism of foreign compounds between mammals and fish is also observed with this drug.

THE PRESENT STUDY

The aim of this work was to expand the current knowledge of the physiological effects, and in particular the cardiovascular effects, of fish anaesthesia using a variety of “whole animal” and *in vitro* techniques. Experiments looking for changes in the whole animal (Chapter 2) were initially performed. The results from these experiments dictated the specifics of the *in vitro* experiments which constitute Chapters 3 and 4. Chapter 5 summarises all of the findings and attempts to form a coherent story.

The anaesthetics examined in this study were MS222, metomidate and Aqui-S. MS222 was chosen because of the large amount of background material available and was used as a type of benchmark. Metomidate was chosen because it seems to have a different mode of action to MS222, has some interesting effects on cortisol production (Brown *et al.*, 1986; Davis *et al.*, 1982; Iwama *et al.*, 1989; Kreiberg, 1992; Kreiberg and Powell, 1991; Limsuwan *et al.*, 1983b; Olsen *et al.*, 1995; Thomas and Robertson, 1991) and has the potential to become a popular aquaculture tool. Aqui-S is a product specifically designed as a fish anaesthetic which, to this author's knowledge, is unique among chemicals used as fish anaesthetics. The constituents of Aqui-S are

food-based which should allow Aqui-S to avoid some of the time consuming and costly tests required before it can be used on food fish. The anaesthetics are described briefly below.

MS222

MS222 (alternative names: tricaine, FinquelTM, metacaine, ethyl *m*-aminobenzoate methanesulphonate) was developed as a mammalian local anaesthetic but was first used to anaesthetise poikilotherms in about 1920. MS222 is a fine, white, crystalline powder that readily dissolves in water (solubility 1 g/0.8 mL). Its high solubility is partially due to it being a salt. Its two parts are methanesulphonic acid (a strong acid) and ethyl *m*-aminobenzoate (a weak base) which causes solutions of MS222 to be acidic. A 100 ppm MS222 solution in deionised water has a pH of two. For this reason MS222 solutions are often buffered with agents such as imidazole, Na₂HPO₄, NaOH or NaHCO₃ before use. In seawater or hard waters buffering may not be necessary. Buffering of concentrated solutions tends to form precipitates and the active unit, ethyl *m*-aminobenzoate, becomes uncharged at higher pH and forms an oily, insoluble liquid. This generally does not occur with dilute solutions. Ethyl *m*-aminobenzoate is more lipid soluble when uncharged so buffering MS222 solutions increases the ability of MS222 to enter tissues and so increases effective concentration of a solution. However, it is the charged form of local anaesthetics that is the active form (McDonald and Wann, 1978). MS222 solutions are unstable in sunlight (Summerfelt and Smith, 1990). The dose for anaesthesia of salmonids is about 100 ppm (Brown, 1988) but effective doses vary between species. It is the only fish anaesthetic authorised for use in the United States of America for use on food fish by the FDA and after exposure fish must be kept alive in clean water for a further 21 days before being processed (Schnick and Meyer, 1986). Restrictions are less stringent in Canada where the withdrawal period is five days (Burka *et al.*, 1997). It is not mutagenic (Yoshimura *et al.*, 1981).

Metomidate

Metomidate (alternative name: hypnodil) is a relatively new fish anaesthetic first used as a hypnotic in mammals and birds (Brander *et al.*, 1982; Mattson and Riple, 1989). Its effect on the cardiovascular system of mammals is small (Hall and Clarke, 1991). It is an imidazole derivative and occurs as a fine, white, crystalline powder, much like MS222. It is also readily soluble in water but does not affect the pH of solutions and therefore does not require the additional cost of a buffering agent. It is more expensive than MS222 but the effective concentration is ten to 20 times lower. The recovery time of fish anaesthetised with metomidate is relatively long (Gilderhus and

Marking, 1987). Metomidate is allowed to be used on food fish in Canada for certain procedures and it is currently in the licensing process in the United States of America (Wedemeyer, 1996). Metomidate has no analgesic properties in mammals (Hall and Clarke, 1991). Spontaneous muscle movement in fish and mammals anaesthetised with metomidate is not uncommon (Hall and Clarke, 1991; Stoelting and Miller, 1989) although it is supposed to possess strong central muscle relaxant properties (Hall and Clarke, 1991). Metomidate reduces the cortisol response in fish and one of its analogues, etomidate, has been shown to block the cortisol synthesis pathway (Olsen *et al.*, 1995).

Aqui-S

Aqui-S is a recently introduced drug developed specifically as a fish anaesthetic by the Seafood Division of Crop and Food, a New Zealand Crown Research Institute. It occurs as a viscous, honey-like liquid with a sweet smell. It appears to dissolve fully at the suggested concentration of 17 ppm but requires good mixing. Solutions of higher concentration form emulsions which precipitate out as thick droplets over time. Aqui-S was developed to help handle fish and, in particular, for rested harvesting (Anonymous, 1995a; Anonymous, 1995b). The result of rested harvesting is a fish with minimal stress levels and exhaustion. The muscle of such fish lasts longer and is of higher quality (Jerrett *et al.*, 1996). The concentration with roughly the equivalent induction properties as 100 ppm MS222 was determined to be 60 ppm Aqui-S. Aqui-S is a mixture of 50% isoeugenol, which makes up 70 - 90% of clove oil (Keene *et al.*, 1998), and 50% of a commercial food emulsifying agent. Clove oil has been tried as a fish anaesthetic recently with promising results (Keene *et al.*, 1998; Munday and Wilson, 1997; Soto and Burhanuddin, 1995). Aqui-S has been used in New Zealand and trialed in Australia with good results (Anonymous, 1996). Recently Aqui-S 2, which is suggested to be an improvement over the original, has become available.

Chapter 2

Cardiovascular and respiratory responses of whole fish during anaesthetic induction and recovery

INTRODUCTION

Observations of the physiological effects of anaesthesia and anaesthetics on whole fish not only show how the whole organism reacts, but they also give clues as to which organs and systems are affected. The experiments in this chapter gather information about changes in the whole fish during and after anaesthesia. The overall effects of anaesthesia are the cumulative sum of: 1) direct anaesthetic effects; 2) secondary effects such as hypoxia and hypercapnia caused by an anaesthesia induced decrease in ventilation; 3) tertiary effects such as acid-base effects due to the low pH of an anaesthetic; and finally 4) human effects such as handling or even the method used to monitor changes. In order to separate these different effects it is useful to measure a variety of parameters and to observe changes caused by anaesthesia under a number of different conditions. Therefore, the effects of anaesthesia with handling and surgery were also investigated to distinguish changes caused by anaesthetic action from the secondary effects caused by the state of anaesthesia and stress.

Acute respiratory responses of fish to chemical inhalation anaesthesia have been measured in a number of studies. Common observations initially include hyperventilation and an increase in oxygen consumption (McFarland, 1959; Summerfelt and Smith, 1990) possibly as a response to the physical presence of some anaesthetics. However, during induction, hypoventilation (Houston *et al.*, 1971a; McFarland and Klontz, 1969) and a decrease in oxygen consumption (Baudin, 1932a; Blahm, 1961; Dixon and Milton, 1978; Ross and Ross, 1984) are observed as a result of anaesthesia. The reduction in oxygen consumption could be due to either a decrease in the rate of oxygen uptake and/or a decrease in metabolism. Early in recovery, hyperventilation and an increase in oxygen consumption due to oxygen debt and/or stress often occurs (Keys and Wells, 1930; Summerfelt and Smith, 1990). Data on the longer term effects of anaesthetics on fish oxygen consumption are sparse. Longer term monitoring of oxygen consumption post-anaesthesia would not only elucidate the changes in oxygen consumption during recovering but would also give an estimate of recovery periods.

All mammalian general anaesthetics affect the cardiovascular system to a certain degree (Hall and Clarke, 1991; Stoelting and Miller, 1989). Local anaesthetics are known to have severe cardiovascular effects in mammals if allowed to enter the circulatory system in sufficient quantities (Hall and Clarke, 1991; Stoelting and Miller, 1989). Changes in blood circulation during anaesthesia may detrimentally effect fish and could be a cause of mortality normally attributed to "over anaesthesia". Cardiovascular effects of fish anaesthetics have been examined previously, mostly with respect to changes in heart rate (Campbell and Davies, 1963; Fredricks *et al.*, 1993; Houston *et al.*, 1971a; Lochowitz *et al.*, 1974; Randall, 1962; Randall and Smith, 1967; Randall *et al.*, 1965; Serfaty *et al.*, 1959) but rarely have blood pressures (Fredricks *et al.*, 1993; Peirce and Peirce, 1967; Randall *et al.*, 1965) or cardiac output (Peirce and Peirce, 1967) been measured. In this study all three variables were measured during induction and recovery from anaesthesia. In addition, cardiac output and heart rate were measured during anaesthesia following handling and during recovery from anaesthesia and surgery.

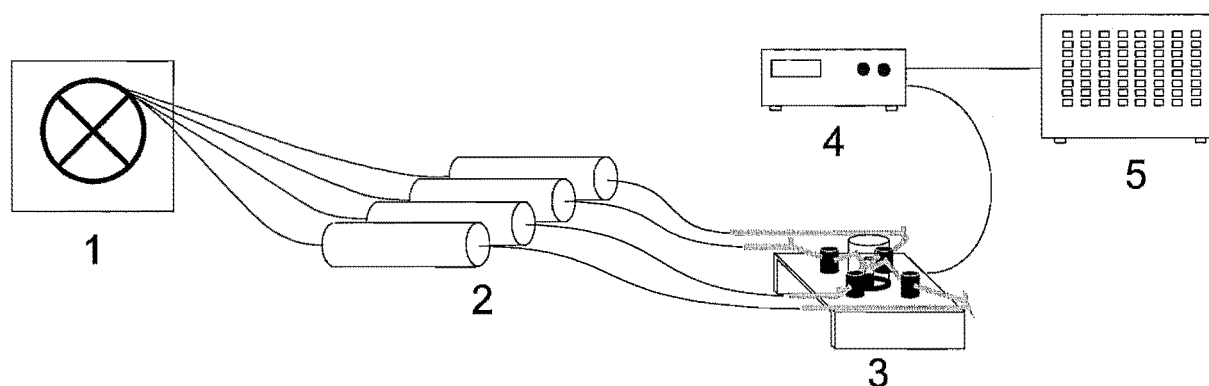
MATERIALS AND METHODS

SERIES 2.1. Long term effects of acute deep anaesthesia on respiration.

Spotties (*Notolabrus celidotus*, mass 34.4 ± 6.3 g (2 SEMs), range 6.7 - 61.9 g, n = 30) were caught in traps in Lyttleton Harbour, South Island, New Zealand and transported to the University of Canterbury Zoology Department. They were placed in a recirculating seawater system (ten - 15 °C, 12 hour/12 hour artificial light/dark regime) and fed mussels (*Perna canaliculus*) two to three times per week. To avoid elevation of respiration due to digestion (specific dynamic action) fish were starved for at least 48 hours prior to each experiment.

The experimental set up is shown in Figures 2.1a&b. Fish were placed in 500 mL flow through tubular respirometers and PO₂ recordings from the expelled water initiated (Strathkelvin Instruments, Model 781, oxygen meter and electrode). Data were collected on a Thurlby (DSA524) digital data storage device and were later downloaded onto a personal computer. The PO₂ differences between expelled water from the respirometers holding fish and from a control respirometer (identical except for the absence of a fish) were used to calculate oxygen consumption by the fish. An automatic valving system directed water from the four respirometers through a single oxygen electrode. Flow rates were controlled using a peristaltic pump and were monitored regularly. These were varied between five and 50 mL.min⁻¹ depending on the size of the fish. At these low flow rates, compared to the volume of the chamber, the set up was unable to detect transient changes in oxygen consumption by the fish, as the time required to reach an

a



b

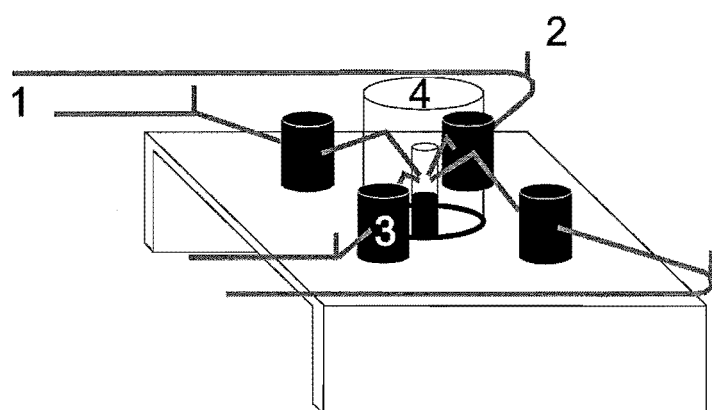


Figure 2.1a&b. Diagram of respirometry experimental set up. Fresh seawater is pumped (a1) into four chambers (a2), three holding fish and the fourth being a control. Outflowing water moves to an apparatus (a3 and b) which measures the PO_2 of the water from each chamber on a rotational basis. Water from each chamber moves down stainless steel pipes (b1) to electronically controlled gates (b3). Only one gate is open at a time. Water from pipes with closed gates flows out of secondary pipes branching off the main pipes (b2). Each gate opens for 10 minutes so an entire sequence takes 40 minutes. Open gates allow water to flow across an oxygen electrode situated within a water jacket (b4). The oxygen meter (a4) data is stored by a Thurlby digital storage device (a5).

equilibrium was quite long. However, for the purposes of this experiment, where a long period of oxygen consumption was measured and longer term changes were being sought, the equipment was suitable. The main drawback was the unreliability of the first recording after setting up the equipment.

After an initial 24 hour monitoring period the fish were anaesthetized by injecting a concentrated solution of one of the three anaesthetics into the respirometers so that the final concentration caused deep anaesthesia of fish within five minutes. The final concentrations, determined prior to the experiment, were 100 ppm MS222 (+300 ppm $NaHCO_3$ to neutralize the MS222), ten ppm metomidate and 60 ppm Aqui-S. After five minutes of exposure to the anaesthetics, the fish were

removed, the respirometers emptied, and the apparatus reassembled for another 24 hours of recording. This method of anaesthetic removal was used as washing out of the anaesthetics with normal flows would have been prolonged, due to the relatively low flow rates, and would have varied between set ups due to the difference in flow rates between set ups. Fish still responsive after five minutes of anaesthetic exposure were fully anaesthetized before being returned to the respirometers. A total of ten fish were exposed to each anaesthetic. The PO_2 of water expelled from each respirometer was measured over ten minute periods in a 40 minute cycle (switching between the four respirometers) for the entire 48 hour period.

Two way ANOVAs (variables being anaesthetic type and time) were performed separately on the -24 to -12 hour oxygen consumption data, the data from -12 hours to immediately prior to anaesthesia, and the 24 hour post-anaesthesia data. These blocks were chosen because the initial 12 hour block should contain fish recovering from handling stress, the second 12 hour block should contain fish respiring at their standard metabolic rate, and the last 24 hour block is the treatment period. The same analysis was performed using data from both the first 12 hour block after setting up the experiment and the first 12 hours after anaesthesia, with the three anaesthetics and handling considered as the four “anaesthetic types”, in an attempt to see if there was a difference between recovery post-handling and post-anaesthesia (ie. the variables were time and anaesthetic x three + handling). The first values post-anaesthesia and post-set up were not used in this analysis due to their unreliability (see above). Primary interactions were tested further using Tukey’s HSD post-hoc analysis. The mean standard metabolic rate of all fish was calculated from the data accumulated in the 12 hours prior to anaesthesia.

For each fish, minimum and maximum post anaesthesia metabolic rate and the time at which the fish’s metabolic rate returned to within its resting value range, were recorded. The effect of anaesthesia on the length of time for fish to return to resting metabolic rates was analysed using ANOVA. ANOVA was not performed on the maxima and minima as these data would be biased by the selection criteria.

Possible differences between weight specific respiration rates of different sized fish were investigated by performing a linear regression on log transformed weight and log transformed respiration rate data, and analysing the resulting line’s slope to see if it was significantly different from zero. Data from the 12 hours prior to anaesthesia were used for this analysis. In all cases $P \leq 0.05$ was used to indicate a significant difference.

SERIES 2.2. Cardiovascular changes and haematology of Chinook salmon during and after anaesthesia.

The order in which the cardiovascular experiments were performed was: recovery from anaesthesia and surgery, stressed induction, unstressed induction and, finally, recovery from anaesthesia. The order in which they are presented was changed in order to group similar experiments and present a clearer story. The order in which they are presented is: unstressed induction, stressed induction for comparison, recovery from anaesthesia and, finally, recovery from anaesthesia plus surgery for comparison.

Initially, ten ppm metomidate was used in the respiration and stressed induction experiments. This concentration was chosen as it produced deep anaesthesia in the fish within about five minutes and was comparable, in this respect, to 100 ppm MS222 and 60 ppm Aqui-S. However, fish anaesthetized with metomidate twitch and can respond to manipulations. As one of the main indicators of deep anaesthesia in fish is loss of skeletal muscle tone, selecting an equipotent concentration of metomidate was difficult and a relatively high concentration was selected. This proved to be too high for surgery in the first cardiovascular experiment as a disproportionately large number of metomidate anaesthetised fish died within 48 hours post-surgery. The concentration was reduced to six ppm, which induced light anaesthesia, allowing fish to recover fully from the surgery, but ten ppm was still used for the measured induction. However, the induction data suggested ten ppm metomidate induces anaesthesia much more quickly than either 100 ppm MS222 or 60 ppm Aqui-S. Therefore, in the second cardiovascular experiment seven ppm metomidate was used for both the surgery and the measured induction as six ppm metomidate only induced light anaesthesia.

The Chinook salmon (*Oncorhynchus tshawytscha*) used in Series 2.2 were purchased from Isaac's Salmon Farm, McLean's Island, Christchurch, and were transported to the University of Canterbury. They were stored in a large (1.5 x 1.5 x 1.5 m), outdoor water tank with constantly flowing fresh artesian water. The tank water and the water supply used in the experiments were from the same source and had an average temperature of 14 °C.

Experiment 2.2a. Measures of cardiovascular and blood variables of Chinook salmon during unstressed anaesthesia.

Chinook salmon (1171 ± 102 g (2 SEMs), range 583 -1636 g, n = 44), when required, were removed from the holding tank with a net and placed in a bucket of the appropriate anaesthetic

until they had reached surgical anaesthesia (gill movement had ceased). The anaesthetics used were MS222 (100 ppm + 300 ppm NaHCO₃), metomidate (seven ppm) or Aqui-S (60 ppm).

Fish were then placed on an operating sling and a Doppler probe (Bioengineering, University of Iowa) was attached to the ventral aorta. The fish were placed on their left side and the right operculum reflected. A small incision (about five mm) was made over the ventral aorta. A syringe needle was inserted in the ventral side below and just anterior to the incision, and pushed through perpendicular to and just below the ventral aorta. A steel spatula was employed to protect the ventral aorta from being punctured with the needle. The Doppler probe lead was threaded down the needle and the needle removed. The probe was placed around the ventral aorta using fine, angle tipped forceps.

A piece of cannula (one mm OD, 0.5 mm ID, about one m long) was then inserted into the dorsal aorta via the roof of the mouth. A syringe needle was used to make a small hole in the tough skin in the midline of the roof of the mouth between the second gill arches. An internal trochar (sharpened 0.483 silver coated guitar wire, Darco strings) in the cannula was pushed through the hole, at a 45° angle, until the tip of the trochar touched the spine. The trochar was then removed sucking blood up the cannula. The cannula was moved further into the dorsal aorta and one mL of heparinized saline solution was slowly injected into the fish. The cannula was exteriorised through the snout via a larger diameter cannula (1.8 mm OD, 1.05 mm ID, one to two cm long). The fish were then placed in the experimental containers (see Figure 2.2 for experimental set up) with a running fresh water supply at 14 °C for 48 hours to recover. The containers were covered with black polythene in an attempt to shield the fish from external visual stimuli which may have disturbed them.

The Doppler flow meter (Model 545C-4, Bioengineering, University of Iowa) measures blood velocity. From these data heart rate and cardiac output were derived. The blood velocity data were collected graphically on a Devices MX4 recorder and digitally on a computer using Labtech software (see Figure 2.2). Dorsal aortic blood pressure (cmH₂O) was measured via the cannula using a pressure transducer (Model 4-327, Bell and Howell Instruments Division, Pasadena, California) and the data collected as above. Blood samples (800 - 900 µL) were also extracted via the cannula. Blood PO₂ was measured using an oxygen meter (Strathkelvin Instruments, Model 781), haematocrit was calculated using microcapillary tubes and a haemofuge, and the remaining blood spun and the plasma collected for catecholamine analysis.

EDTA/reduced glutathione (25 µL, 0.2 M / 0.2 M) was added to the plasma for catecholamine analysis and was then stored at -70 °C. When required, 300 µL plasma was added to 500 µL

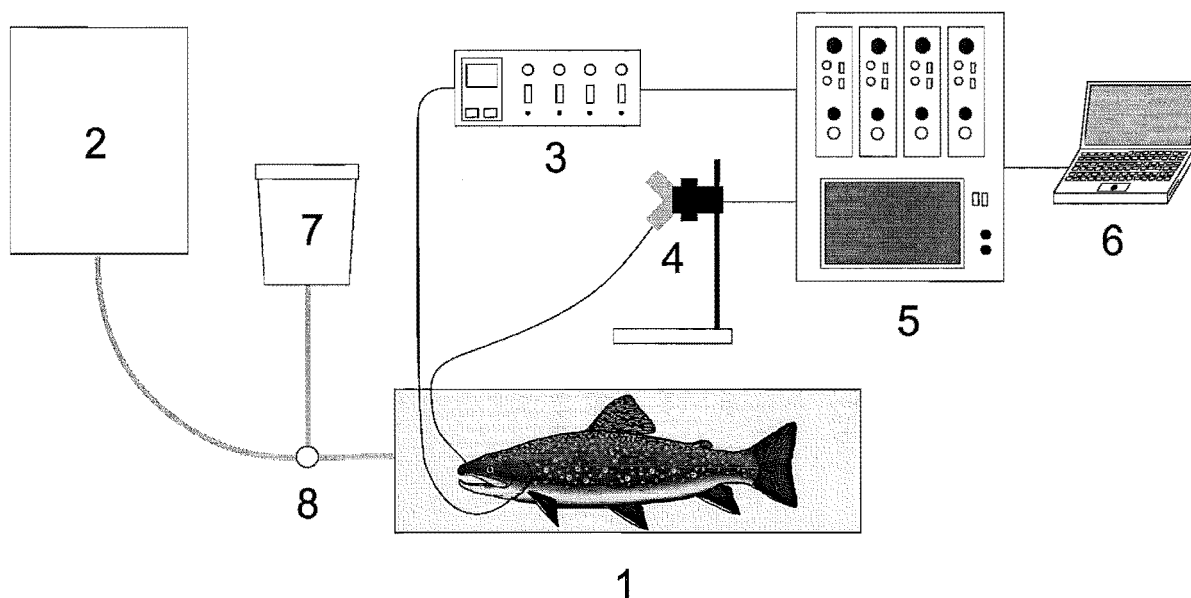


Figure 2.2. Diagram of experimental set up for Series 2.2 experiments. After surgery, fish were placed in the recovery/experimental box (1). The box was supplied with constantly flowing freshwater from a reservoir (2). The Doppler flow probe was attached to the Doppler meter (3). The dorsal aortic cannula (if fitted) was attached to a pressure transducer (4). Data from the Doppler meter and pressure transducer was graphically recorded on a Devices MX4 recorder (5) and digitally on a laptop computer (6). When required an anaesthetic solution from a bucket (7) could be exchanged for the freshwater supply (2) using a tap (8).

TRIS/EDTA (1 M / 50 mM) with about ten mg alumina. This was mixed thoroughly for 15 minutes (to allow the catecholamines to adsorb onto the alumina), spun, and the supernatant removed. The alumina was washed three times for five minutes with 500 μ L double distilled water (ddH_2O). After each wash the contents were spun and the supernatant removed. Finally, the alumina was mixed with 150 μ L acetic acid (0.2 M) for five minutes (to elute the catecholamines off the alumina) and then centrifuged. The supernatant was injected into a high performance liquid chromatograph (HPLC, Shimadzu, column: C18, five μ m, 4.6 mm diameter, 250 mm length, 30 $^\circ\text{C}$) with an electrochemical (EC) detector (L-ECD-6A, +0.6 V). The mobile phase¹ was pumped through the column at one $\text{mL}\cdot\text{min}^{-1}$. Noradrenaline and adrenaline peaks came through at about 4.7 and 5.9 minutes respectively. A standard curve was made to calculate exact concentrations. Standards were made from L-noradrenaline bitartrate (Koch-Light Laboratories Ltd.) and adrenaline hydrochloride (SIGMA) and were treated and stored as the plasma samples.

After the 48 hour recovery period measurements were taken during a subsequent anaesthetic induction. Anaesthetic solutions were introduced via the same inflow line as the normal water supply. Switching between anaesthetic solution and normal water supply was performed by

¹ The mobile phase consisted of 7.941 g tri-sodium citrate, 0.034 g EDTA and 0.135 g heptane sulphonic acid dissolved in 900 mL ddH_2O . To this acetic acid was added to achieve pH 5.00 and then 100 mL of methanol was added.

switching a three-way tap situated about one m away from the fish boxes. The flow rates of the anaesthetic solution and normal water supply were slightly different and on occasion there was a slight temperature difference between the anaesthetic solutions and the normal water supply. Prior to induction a blood sample was taken and replaced with an equal volume of freshwater fish Ringer's solution. Induction consisted of a ten minute period where heart rate, cardiac output and dorsal aortic pressure were recorded constantly. Stroke volume was calculated from cardiac output and heart rate data (Q/HR). The first five minutes were used as a control and over the next five minutes the anaesthetics were introduced. The anaesthetics and concentrations used were the same as used for the surgery. After the induction period a second blood sample was taken as above. Data were also collected from these fish for Experiment 2.2c over the next 48 hours.

Experiment 2.2b. Measures of cardiovascular variables of Chinook salmon during a stressful anaesthetic induction.

Chinook salmon (292 ± 23 g (2 SEMs), range 155 - 382 g, $n = 21$), when required, were removed from the tank with a net and placed in a bucket of the appropriate anaesthetic until they had reached surgical anaesthesia. The anaesthetics used were MS222 (100 ppm + 300 ppm Na_2CO_3), metomidate (six ppm) or Aqui-S (60 ppm).

Fish were then placed on an operating sling and a Doppler probe was attached to the ventral aorta. The procedure was similar to that described in Experiment 2.2a but the needle was inserted from the opposite side. The needle was inserted into the skin of the body wall just posterior to the operculum, and pushed underneath the skin so that the needle emerged perpendicular to and just above the ventral aorta. Data were recorded as above. Recovery data were collected from these fish for Experiment 2.2d over the next 48 hours.

After 48 hours the fish were re-anaesthetized in the same anaesthetics by removing the fish from their boxes and placing them in a bucket of anaesthetic. The concentrations were the same as for the surgery except for metomidate where ten ppm was used instead of six ppm. Recording of data was initiated within 1.5 minutes. This time was required in order for the fish to become quiescent enough to attach leads to the Doppler probe. Blood velocity data were recorded continuously until 15 minutes post-induction. After the experiment, cardiac output was calibrated by pumping a blood solution (one part blood + five to ten parts Ringer) into the *bulbus arteriosus* and measuring the resulting Doppler values from the ventral aorta. This was performed on dead animals with the heart *in situ*. The resting cardiac output (ie. $Q = 1$) was calculated to be 14 ± 1 mL.min⁻¹.kg⁻¹ (2 SEMs).

Experiment 2.2c. Measures of cardiovascular variables of Chinook salmon during recovery from unstressed anaesthesia.

The fish used in this experiment were those used in Experiment 2.2a. After the second induction (after surgery and 48 hours of recovery) measurements of heart rate, cardiac output and dorsal aortic pressure were recorded at five, ten, 15, 30, 45 minutes, one, two, three and six hours post-anaesthesia.

Experiment 2.2d. Measures of cardiovascular variables of Chinook salmon during recovery from anaesthesia and surgery.

The fish used in this experiment were those used in Experiment 2.2b. Recordings of heart rate and cardiac output were taken at five, ten, 15, 30 minutes one, two, three, six, 12, 24, 36 and 48 hours following the initial anaesthesia and surgery.

For all Series 2.2 experiments, heart rate, cardiac output and dorsal aortic pressure data (where measured) were compared using repeated measures ANOVA. Tukey's HSD test or its equivalent for uneven n values was used to determine specific differences if significant primary effects or interactions were found. The control value in Experiments 2.2a and 2.2c was the mean of the five minutes of recording prior to induction in 2.2a, and the control for 2.2b and 2.2d the average of the 36 and 48 hour values from Experiment 2.2d. The PO_2 , haematocrit and catecholamine data from Experiment 2.2a were compared using ANOVA (non-parametric in the case of the catecholamines and PO_2). $P \leq 0.05$ was used as the level of significance in all cases.

SERIES 2.3. Salmon blood oxygen equilibrium curve and P_{50} .

Oxygen equilibrium curves were plotted from the blood of eight salmon. Chinook salmon blood was mixed with humidified gas mixtures (0.5 % CO_2 , remainder made up of N_2 and O_2) provided by a gas mixer (GF-3 gas mixing flowmeter, Cameron Instrument Company, Texas) in a spinning tonometer (DEQ-1 dual equilibrator, Cameron Instrument Company, Texas) at 13 °C. Blood PO_2 was measured using a Strathkelvin Instruments Model 781 oxygen meter and oxygen content was measured using an oxygen content analyser (Oxy Con, Physiology Department, University of Tasmania). From the plotted curves (fitted using Hill plot) the P_{50} was estimated and the mean P_{50} calculated. This value was compared to the PaO_2 of the fish blood from Experiment 2.2a to see if there was any correlation between blood PO_2 and release of catecholamines.

RESULTS

SERIES 2.1. Long term effects of acute deep anaesthesia on respiration.

There was no correlation between log fish weight and log respiration rate ($r^2 = 0.0004$) and the slope of the regression line was not significantly different from zero. If it is assumed that weight specific metabolic rate does change with fish size, these data show that variability from unknown factors outweighs the effect of mass on the weight specific metabolic rate over the limited fish size range studied. Therefore, raw data did not require standardising by correcting for scaling effects.

The respiration data are plotted in Figures 2.3a-c. During the initial stages of the first 12 hours there was a decrease in respiration rate with time. The initial elevation in respiration rate was probably due to the stress caused by handling of the fish during setting up of the experiment. Handling effects lasted at least three hours. All of the -12 to zero hour data were the same showing that the fish had recovered from handling and were therefore assumed to be respiring at their standard metabolic rates. The mean standard metabolic rate for all of the animals over this period was $1.88 \pm 0.22 \text{ mmol.kg}^{-1}.\text{hr}^{-1}$ (2 SEMs, $n = 30$).

During the recovery from anaesthesia there was a significant interaction between the anaesthetic used and time. The post-hoc test showed that metomidate treated fish were using less oxygen than MS222 or AQUI-S treated fish over the first one to two hours of recovery and that the mean respiration rate of the MS222 treated fish significantly exceeded their mean standard metabolic rate between about one and 1.5 hours post-anaesthesia. This suggests MS222 treated fish were recovering from an oxygen debt, as their respiration rates were elevated during recovery, and that AQUI-S and metomidate treated fish did not have a significant oxygen debt. However, this method of analysis assumes that all fish anaesthetised with a particular anaesthetic become capable of ventilating efficiently at the same time (time dependent) and this is not the case as there is variation between individuals. If maximum and minimum respiration rates for individual fish are averaged, regardless of when the maxima and minima occurred (time independent), and compared to the mean standard metabolic rates of all fish (calculated from the 12 hours of data collected prior to anaesthesia) the results can be interpreted differently. The maximum time independent respiration rates of all groups were almost twice the standard metabolic rate which suggests all fish incurred an oxygen debt (Table 2.1). The maximum respiration rates of the MS222 and AQUI-S treated fish generally occurred earlier than the maximum respiration rate of the metomidate treated fish, and the time that the respiration rate maxima of the metomidate treated fish occurred tended to be more variable. This resulted in the low and broad mean respiration peak later in the time dependent data of the metomidate treated fish (Figure 2.3b) and the thinner and higher time dependent

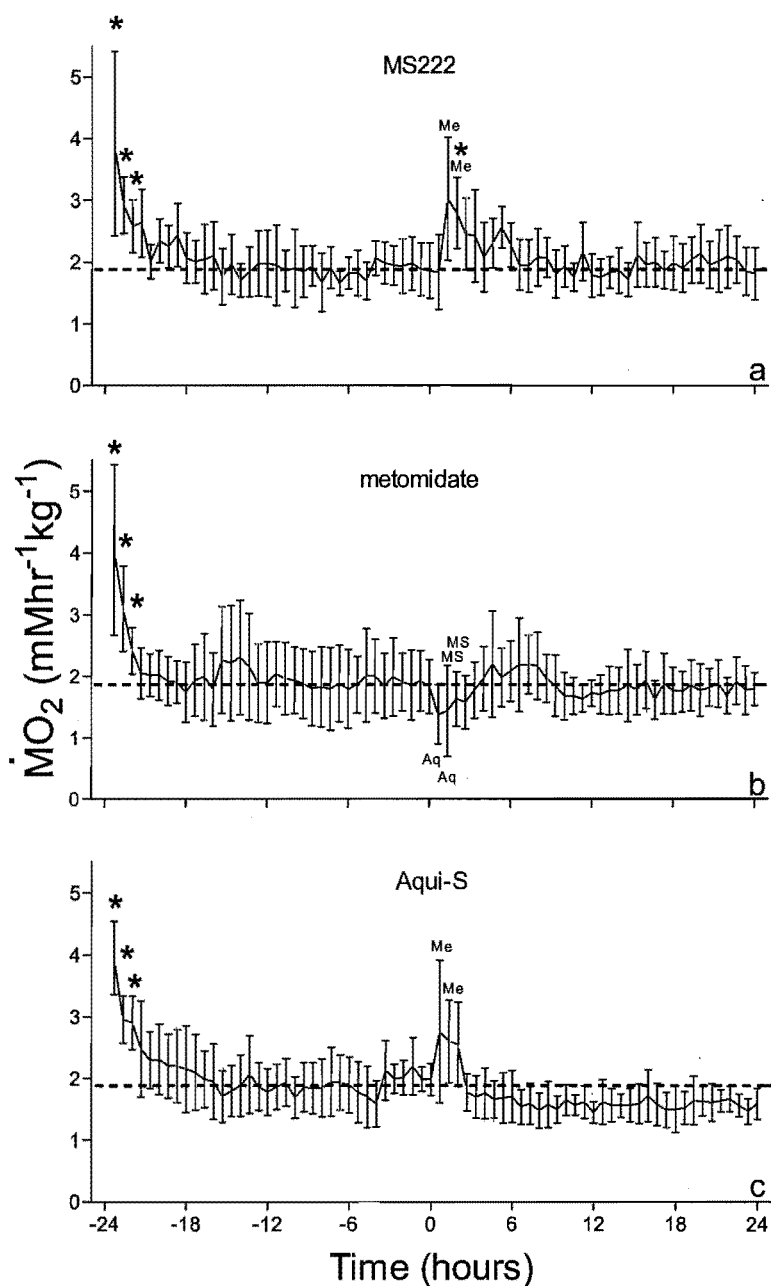


Figure 2.3a-c. Graphs of respiration rate of *P. celidotus* versus time for 24 hours before and after anaesthesia with: (a) 100 ppm MS222 (+ 300 ppm NaHCO₃), (b) ten ppm metomidate, (c) 60 ppm Aquí-S. Data points are means \pm 2SEMs. $n=10$ for each point. Dashed line equals mean $\dot{M}O_2$ of all fish over the 12 hours pre-anaesthesia. * = significantly different from resting. ^{MS}, ^{Me} and ^{Aq} = significantly different from MS222, metomidate, Aquí-S treated fish, respectively, at the equivalent times.

respiration rate peaks of the MS222 and Aquí-S treated fish which occurred earlier in the recovery (Figure 2.3a&c). This suggests that all fish incurred an oxygen debt and that a greater amount of time had elapsed post-anaesthesia before the metomidate treated fish could repay that debt. The minimum time independent respiration rates for MS222 and Aquí-S treated fish were similar to the standard metabolic rate whereas the minimum time independent respiration rate of the metomidate

Table 2.1. Measures of recovery from anaesthesia. Time to normal is the time from initiation of recovery (removal of anaesthetic) to the start of normal respiration. Min. and Max. MR's are the means of the minimum and maximum metabolic rate's of each fish during recovery. Control values are the standard metabolic rates for all fish calculated from data collected for 12 hours prior to anaesthesia. Errors are ± 2 SEMs and n = ten for each value except controls where n = 30.

comparison \ treatment	MS222	metomidate	Aqui-S	Control
Time to normal (hr)	2.8 \pm 0.5	4.7 \pm 2.0	2.5 \pm 0.9	n/a
Min. MR (mmol.kg ⁻¹ .hr ⁻¹)	1.8 \pm 0.6	0.9 \pm 0.3	1.6 \pm 0.4	1.9 \pm 0.2
Max. MR (mmol.kg ⁻¹ .hr ⁻¹)	3.9 \pm 0.8	3.5 \pm 0.8	3.6 \pm 0.9	1.9 \pm 0.2

treated fish was less than half of the standard metabolic rate (Table 2.1). The minimum metomidate values all occurred early in the recovery. This suggests that the ability of metomidate treated fish to extract oxygen was impaired early in the recovery and that these fish were still under the effect of metomidate. Although there was an oxygen deficit in the MS222 and Aqui-S treated fish, indicated by the increased respiration rate during recovery, a reduced oxygen consumption was not observed and may have occurred and finished prior to accurate recording (due to delay in equilibration of the flow through respirometers). This assumes that the oxygen debt was caused by a reduced oxygen uptake and not an increased oxygen requirement. However, these data do not elucidate how the oxygen debt was effected although oxygen debt must be caused by oxygen uptake not meeting oxygen consumption. Therefore, the cause must be a reduced oxygen uptake and/or an elevated oxygen consumption. There was no statistically significant difference between the anaesthetics in the mean time it took fish to return to their standard metabolic rates. The recovery time of some metornidate treated fish was high which raised the error value of the mean (Table 2.1).

The results from the statistical comparison of the handling (first 12 hour period) and anaesthesia (third 12 hour period) recovery data showed that there was a significant interaction between time and anaesthetic/handling. The post-hoc test showed that there were one (MS222 and Aqui-S) or two (metomidate) times at which the anaesthesia recovery respiration rates were significantly lower than the handling recovery data. This suggests that handling caused a greater effect on respiration than anaesthesia.

SERIES 2.2. Cardiovascular changes and haematology of Chinook salmon during and after anaesthesia.

For all Chinook salmon used in the Series 2.2 experiments the mean resting heart rate was 50.8 ± 3.1 beats per minute (2 SEMs, $n = 63$) and the mean resting dorsal aortic blood pressure was 46.3 ± 1.8 cmH₂O (2 SEMs, $n = 41$) which is equal to 34.0 ± 1.4 mmHg.

Experiment 2.2a. Measures of cardiovascular and blood variables of Chinook salmon during unstressed anaesthesia.

Heart rate, cardiac output, dorsal aortic pressure and stroke volume data prior to and during induction with the three anaesthetics and a control can be seen in Figures 2.4a-p. Plasma catecholamine, blood PO₂ and haematocrit data are plotted in Figures 2.5a-d.

Occasionally, in some of the control fish, an initial decrease in heart rate accompanied by a slight decrease in cardiac output and a slight increase in dorsal aortic pressure were observed. These changes were thought to be due to a mild response to an alteration in water flow or temperature in the chamber caused by changing the water source. A few fish in all treatments reacted in this manner but they did not cause any significant changes to the means.

MS222 treated fish showed no significant change in heart rate, cardiac output or stroke volume (Fig. 2.4a, b&d) although there were some slight deviations or increases in variation. Dorsal aortic pressure fell constantly during the induction and the fall became statistically significant at about 2.5 minutes (Fig. 2.4c). Dorsal aortic pressure had not reached a plateau by the end of the exposure. Blood PO₂ after five minutes induction was lower than pre-induction (Fig. 2.5c). No other haematological parameters changed during the exposure (Fig. 2.5a,b&d).

Metomidate treated fish showed no significant change in heart rate (Fig. 2.4e) or cardiac output (Fig. 2.4f) although there were small deviations and the variability increased. Stroke volume remained constant (Fig. 2.4h) while dorsal aortic pressure fluctuated but never changed enough to be significantly different from pretreatment values (Fig. 2.4g). There was a significant drop in the PO₂ of blood from the dorsal aorta (Fig. 2.5c) but no other changes in blood variables were detected (Fig. 2.5a,b&d).

Aqui-S treated fish initially showed a significant drop in all four cardiovascular variables (Fig. 2.4i-l). All animals showed marked activity at this time in contrast to the other two treatments and the

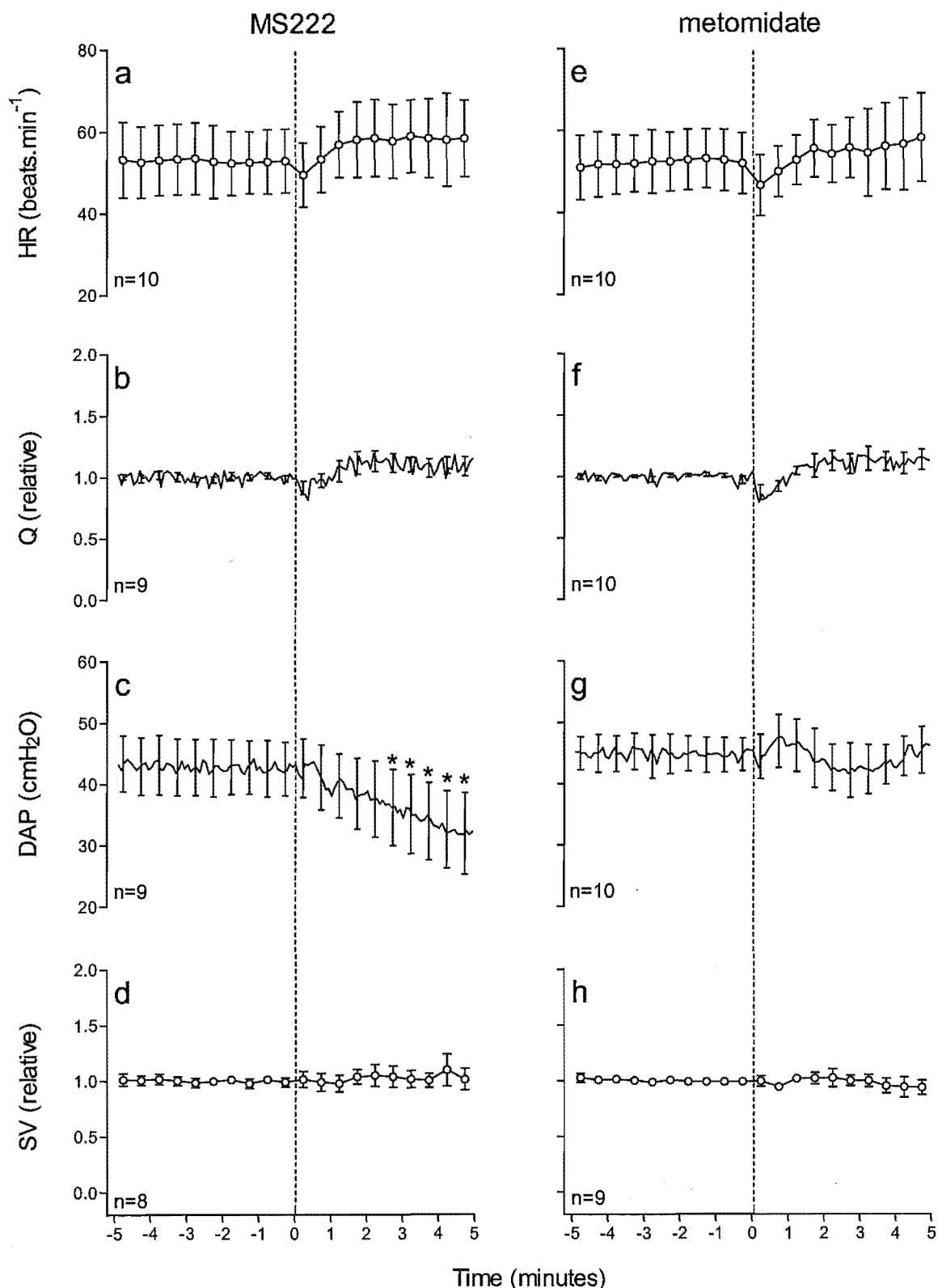


Figure 2.4a-h. Graphs of heart rate (HR), cardiac output (Q), dorsal aortic blood pressure (DAP) and stroke volume (SV) of Chinook salmon during induction of anaesthesia with MS222 and metomidate. Error bars are ± 2 SEMs. * represents a significant difference ($P \leq 0.05$) from pre-induction values. Note that the lines on the cardiac output and dorsal aortic pressure graphs are from data measured every five seconds whereas the error bars are for a 30 second period. The data points and error bars on the induction heart rate graphs are both at 30 second intervals.

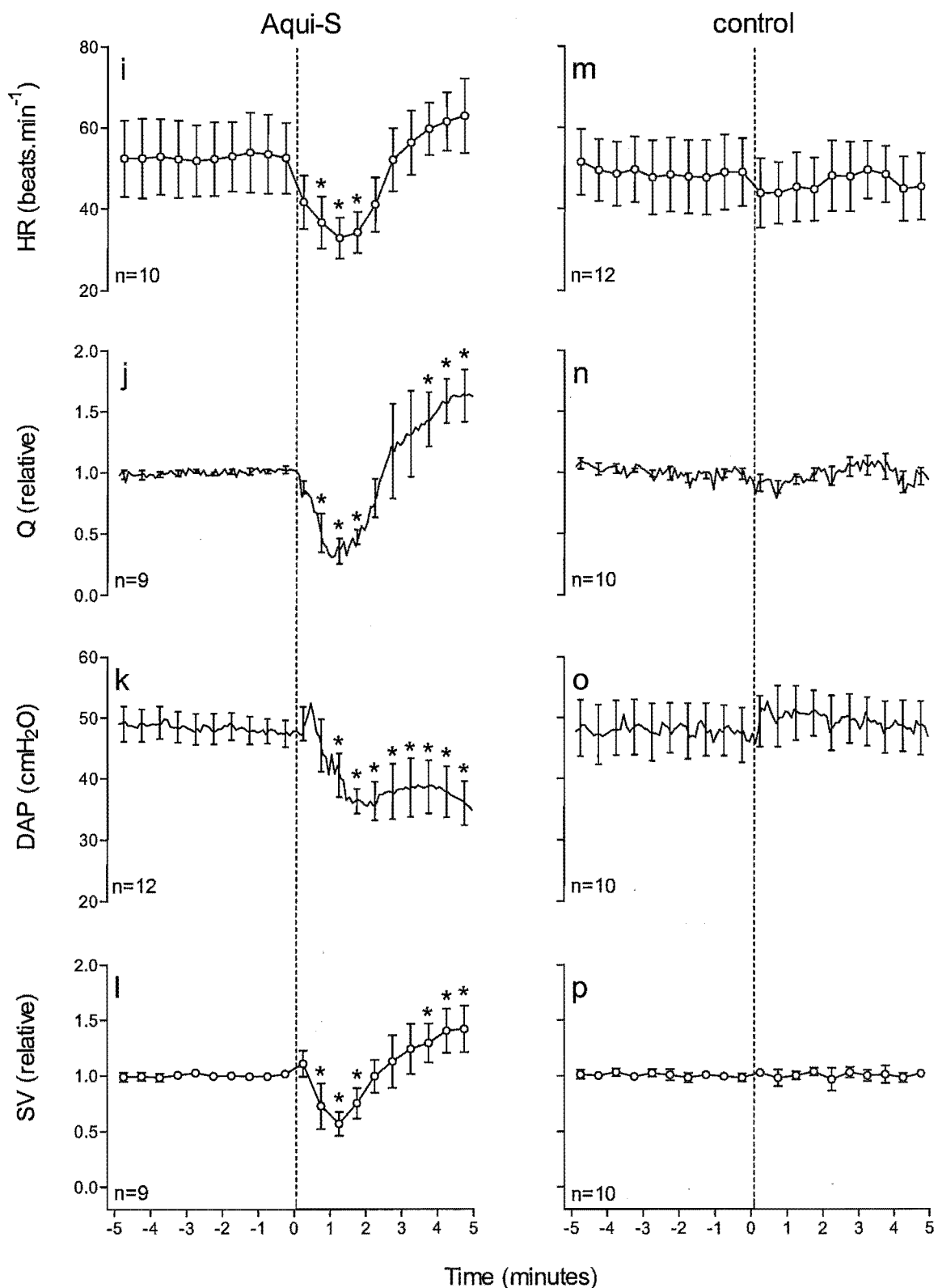


Figure 2.4i-p. Graphs of heart rate (HR), cardiac output (Q), dorsal aortic blood pressure (DAP) and stroke volume (SV) of Chinook salmon during induction of anaesthesia with Aquic-S and for a control where no anaesthetic was used. Error bars are ± 2 SEMs. * represents a significant difference ($P \leq 0.05$) from pre-induction values. Note that the lines on the cardiac output and dorsal aortic pressure graphs are from data measured every five seconds whereas the error bars are for a 30 second period. The data points and error bars on the induction heart rate graphs are both at 30 second intervals.

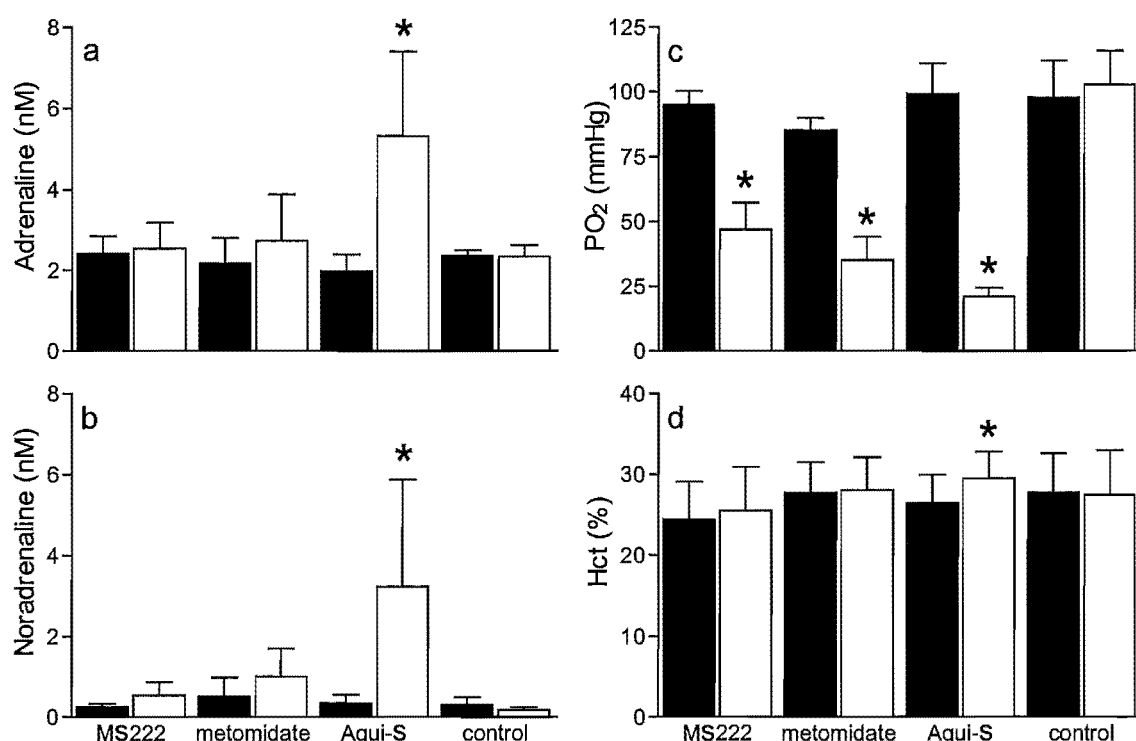


Figure 2.5a-d. Graphs of adrenaline concentration (a, $n = 7 - 16$), noradrenaline concentration (b, $n = 7 - 13$), blood PO_2 (c, $n = 8 - 11$) and haematocrit (d, $n = 8 - 13$) of the blood of Chinook salmon before (black) and after (white) five minutes exposure to the anaesthetics stated. Control is water applied in the same manner as the anaesthetics (see text). * denotes a significant difference between pre-exposure and post-exposure ($P \leq 0.05$). Error bars are ± 2 SEMs.

control fish. By three minutes heart rate (Fig. 2.4i), cardiac output (Fig. 2.4j) and stroke volume (Fig. 2.4l) had returned to within the resting range. Cardiac output and stroke volume increased to higher than pre-induction levels by 4.5 minutes (Fig. 2.4j&l). Heart rate, cardiac output and stroke volume started increasing at about two minutes after exposure which suggests a single process was affecting these three parameters. After the initial fall dorsal aortic pressure remained low, however, the variation increased possibly obscuring any further changes (Fig. 2.4k). Blood PO_2 fell during exposure to Aquí-S, as with the other anaesthetics (Fig. 2.5c), but, unlike the other two anaesthetics, haematocrit and plasma adrenaline and noradrenaline levels increased (Fig. 2.5a,b&d).

Experiment 2.2b. Measures of cardiovascular variables of Chinook salmon during stressful anaesthetic induction.

The heart rate, cardiac output and stroke volume of fish subjected to capture and handling stress and anaesthetic induction are plotted with the data from the previous experiment (Fig. 2.6a-i).

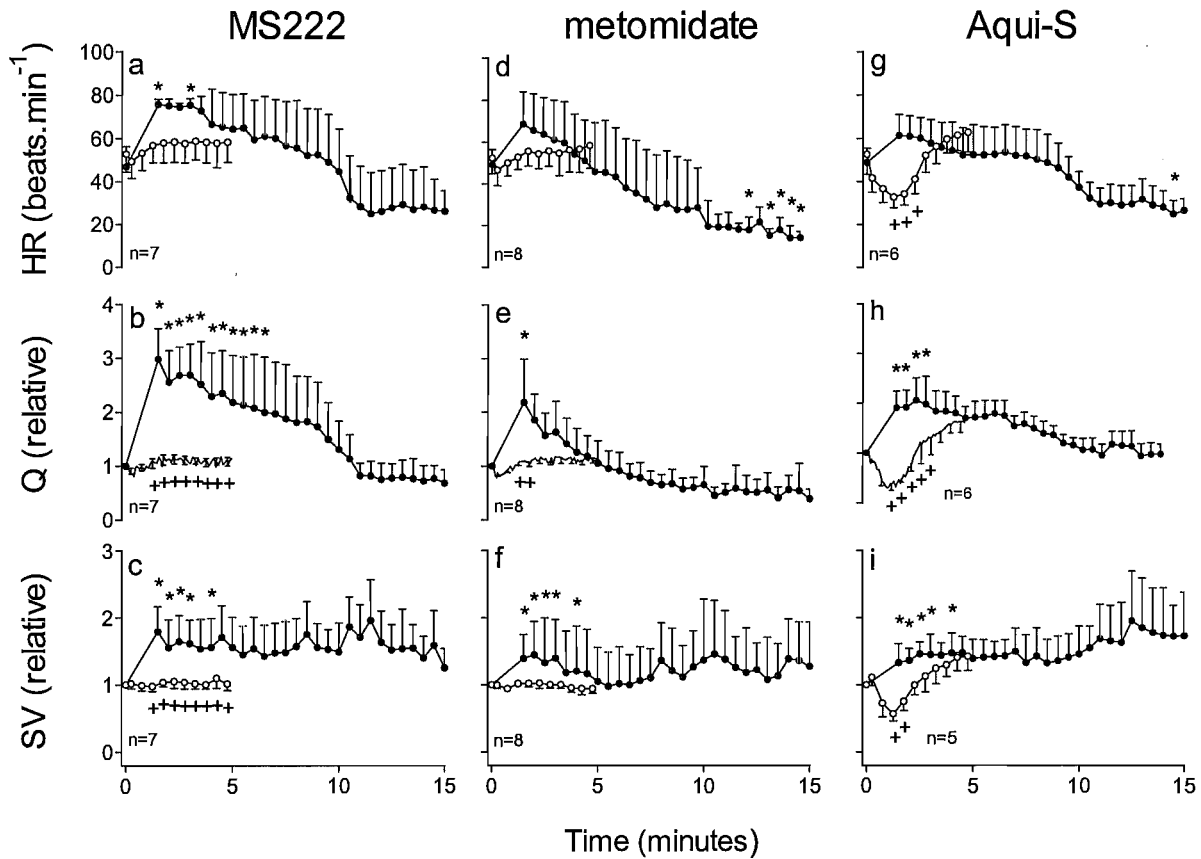


Figure 2.6a-i. Graphs comparing heart rate (HR), cardiac output (Q) and stroke volume (SV) of Chinook salmon during stressed (●) and stress free (○ or no symbol) induction of anaesthesia. n values are for the stressed data. n values for the unstressed data are shown in Figure 2.4. Error bars are ± 2 SEMs. * designates a significant difference ($P \leq 0.05$) of the stressed anaesthesia values from the pre-anaesthesia values (time = 0). + designates a significant difference between the anaesthesia recovery and the anaesthesia and surgery recovery data.

Recording of cardiovascular variables could not be initiated immediately upon immersion in the anaesthetics due to the struggling of the fish. Data are shown from 1.5 minutes after immersion. In general, all anaesthetic treatments showed an elevated heart rate, cardiac output and stroke volume when recording was initiated although not all of these increases were statistically significant. These increases were most likely due to the capture and handling stress rather than the anaesthesia as these effects were not seen in the previous experiment. Heart rate and cardiac output then decreased rapidly while stroke volume remained relatively constant. Statistically there was an interaction between anaesthetics and time for heart rate and cardiac output, but only time was a significant factor in determining stroke volumes. The increase in heart rate for MS222 treated fish (Fig. 2.6a) was greater and the variability smaller than either the Aquic-S (Fig. 2.6d) or metomidate (Fig. 2.6g) treated fish. This produced a higher cardiac output in MS222 treated fish. Note that the heart rate and cardiac output of the metomidate treated fish (Fig. 2.6e) dropped faster

than those of the other two anaesthetic treated fish (Fig. 2.6b&h). As mentioned previously, this was probably due to a disproportionately high concentration of metomidate used in this experiment.

There were a number of differences between the data from Experiments 2.2a and 2.2b. The differences in cardiac output and stroke volume of MS222 and metomidate treated fish were probably due to stress alone. The transient falls in heart rate, cardiac output and stroke volume seen in the Aqui-S treated fish in Experiment 2.2a were not observed in Experiment 2.2b but the increase in these variables in Experiment 2.2b were not as great as the increases seen for MS222 and metomidate treated fish. The effects of stress and Aqui-S may have been competing to produce the intermediate values observed.

Experiment 2.2c. Measures of cardiovascular variables of Chinook salmon during recovery from unstressed anaesthesia.

Heart rate, cardiac output, dorsal aortic pressure and stroke volume data from fish recovering from five minutes exposure to the anaesthetics and from control fish (water flow from alternative source) can be seen in Figures 2.7a-l. Heart rate, cardiac output and dorsal aortic pressure all changed with time but there were no differences in effect between treatments. Heart rate values at five minutes were significantly different from values between 15 and 45 minutes, but values at all times were not significantly different from control values (Fig. 2.7a,e,i). This suggests an initial transient (< ten minutes) increase in heart rate followed by a slightly depressed heart rate for up to one hour. Cardiac output at six hours was significantly different from pre-anaesthesia controls (Fig. 2.7b,f,i). Dorsal aortic pressure exceeded control values for at least 15 minutes post- anaesthesia and then gradually decreased over the recovery period until at six hours it was significantly lower than control data (Fig. 2.7c,g,k). Note that the dorsal aortic pressure of MS222 and Aqui-S treated fish had fallen by about 25 % by the end of the five minute exposure period so the increase post-anaesthesia was quite large (20 - 25 cmH₂O). There was a significant interaction between anaesthetic and time for the stroke volume data (Fig. 2.7 d,h,l). The only significant effect shown by the post-hoc test was an increase at 30 minutes in the Aqui-S treated fish (Fig. 2.7l). It is likely that recovery was not complete within the six hour time span for any of the anaesthetics as dorsal aortic pressure and cardiac output had not stabilized.

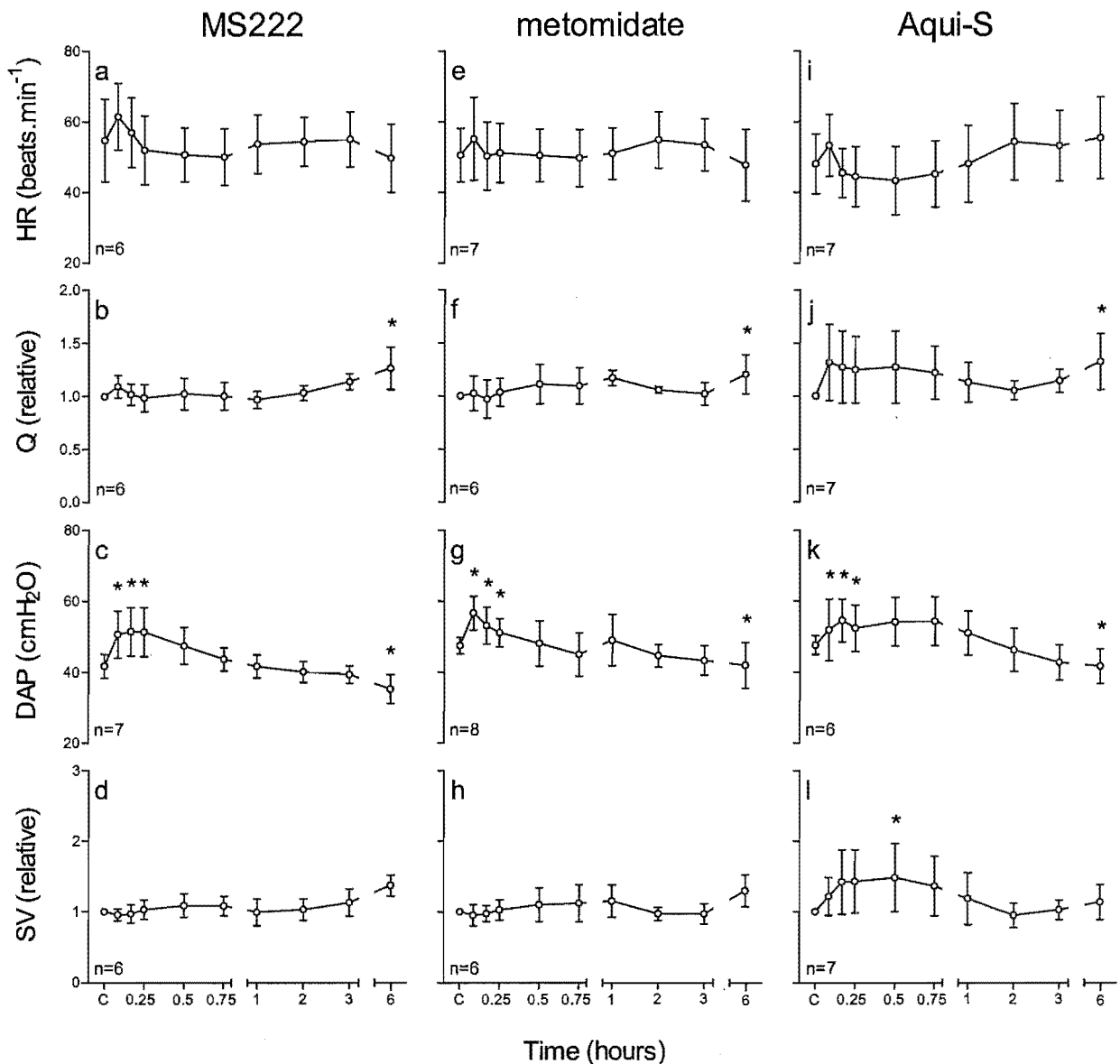


Figure 2.7a-l. Graphs of heart rate (HR), cardiac output (Q), dorsal aortic blood pressure (DAP) and stroke volume (SV) of Chinook salmon during recovery from anaesthesia. Error bars are ± 2 SEMs. * represents a significant difference ($P \leq 0.05$) from control values (C, recorded pre-induction).

Experiment 2.2d. Measures of cardiovascular variables of Chinook salmon during recovery from anaesthesia and surgery.

The heart rate, cardiac output and stroke volume during recovery from anaesthesia and surgery are plotted with the data from Experiment 2.2c in Figures 2.8a-i. The statistical analysis of the heart rate data showed there was a significant interaction between anaesthetic and time. The post-hoc test found no differences between any time during recovery and heart rate pre-anaesthesia for any of the anaesthetics. However, all of the anaesthetic treatments showed significant differences

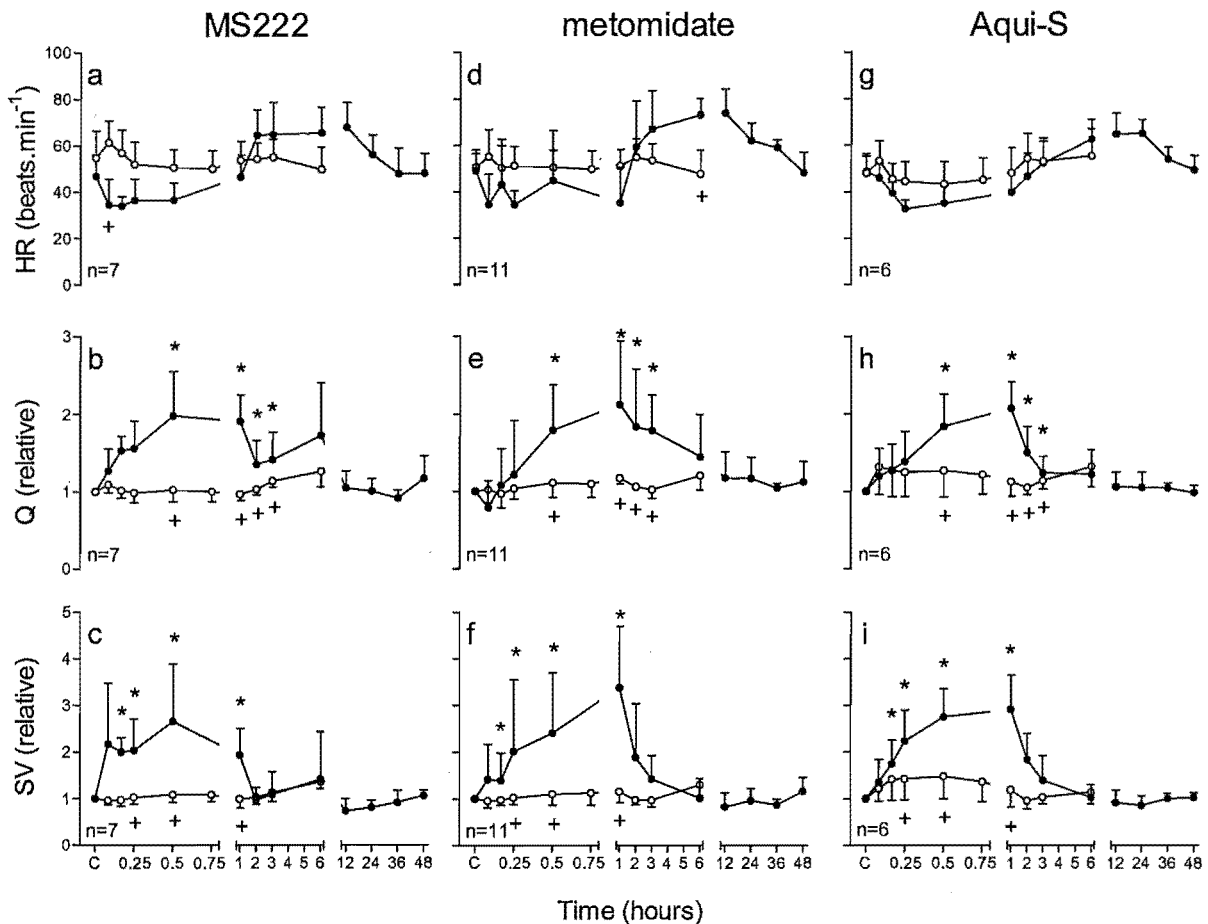


Figure 2.8a-i. Graphs comparing heart rate (HR), cardiac output (Q) and stroke volume (SV) of Chinook salmon during recovery from anaesthesia (○) or anaesthesia and surgery (●). n values are for the post surgery data. n values for the anaesthesia alone data are shown in Figure 2.7. Error bars are ± 2 SEMs. * designates a significant deviation of the anaesthesia and surgery recovery line from its control value (C). + designates a significant difference between the anaesthesia recovery and the anaesthesia and surgery recovery data.

between some of the times during recovery. In general heart rates between five minutes and 60 minutes were lower than heart rates between two hours and 24 hours. The exact times varied between anaesthetics (MS222: five - 30 minute heart rates lower than two to 12 hour heart rates, metomidate: five to 60 minutes lower than three to 24 hours, Aqui-S: ten to 60 minutes lower than six to 24 hours). These differences suggest that for all anaesthetic treatments there is initially a decrease in heart rate for up to one hour followed by an increase in heart rate for up to 24 hours. The cardiac output and stroke volume data only showed an effect of time with cardiac output elevated between 30 minutes and three hours and stroke volume elevated between ten minutes and 60 minutes. Overall, the effects tended to be very similar between treatments which suggests the major causative effect was the surgery rather than the type of anaesthetic used.

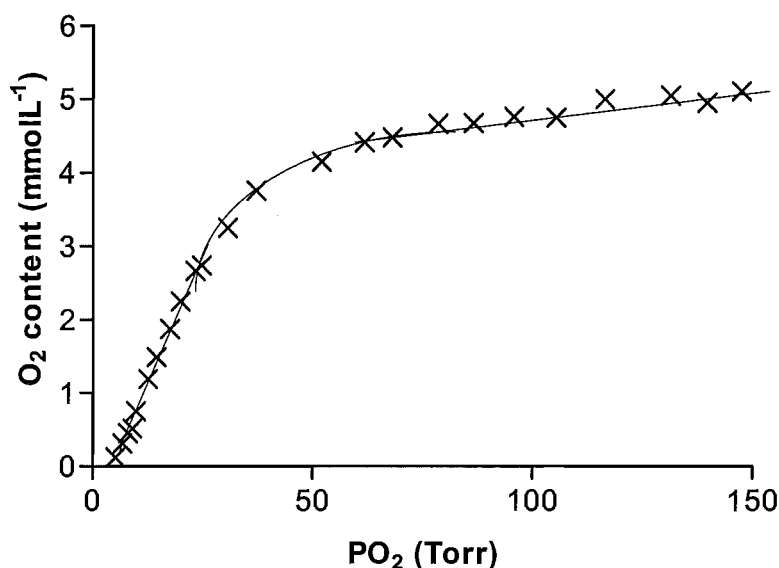


Figure 2.9. A representative oxygen binding curve of the whole blood from *Oncorhynchus tshawytscha*. The line was plotted by hand (Note: Hill plot was used to find P_{50}).

Comparing the data from Experiment 2.2c to these data strongly suggests there is a larger difference between the two experiments than between the anaesthetics within each experiment. This implies that surgery had a much greater effect than any of the anaesthetics and this is reflected in the cardiovascular variables during recovery.

SERIES 2.3. Salmon blood oxygen equilibrium curve and P_{50} .

The P_{50} of Chinook salmon blood was calculated to be 26.3 ± 1.9 torr (2 SEMs, $n = 8$). A representative data set is plotted in Figure 2.9. Plots of plasma adrenaline and noradrenaline concentration versus PaO_2 are plotted in Figure 2.10a&b respectively. Lines marking about 26 Torr are drawn on these graphs allowing a comparison of when catecholamines are released versus the P_{50} of Chinook salmon blood. There were no elevated catecholamine levels occurring at PaO_2 values above the P_{50} but not all blood samples with a PaO_2 below P_{50} had elevated catecholamines.

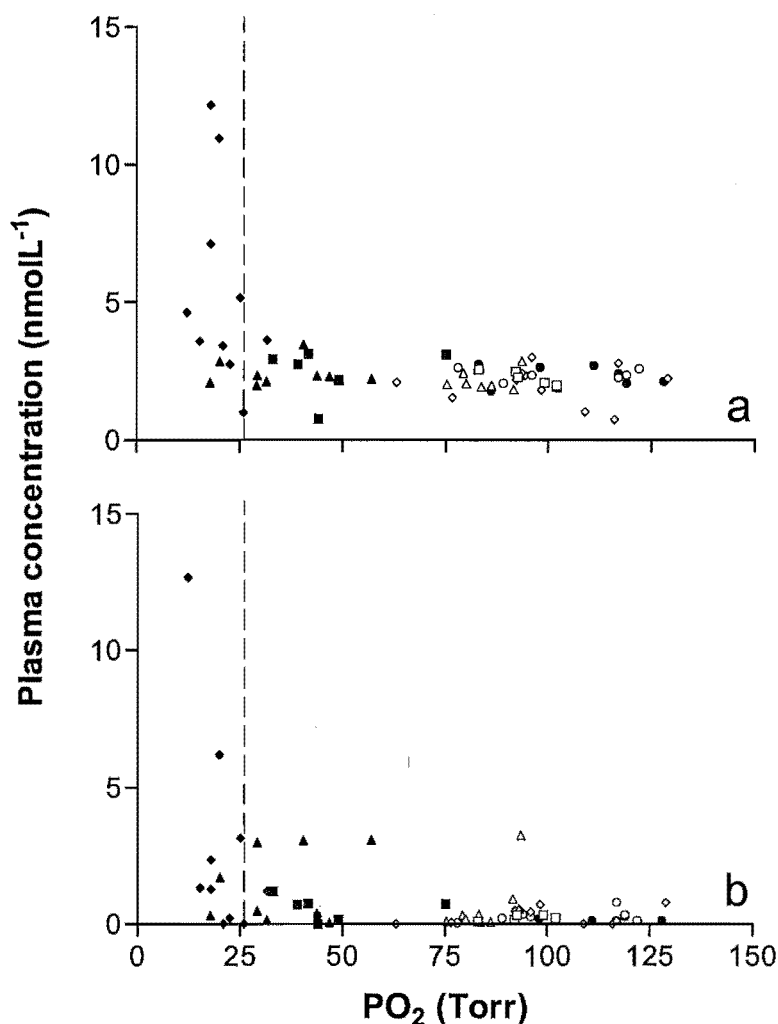


Figure 2.10a&b. Graphs of plasma adrenaline (a) and noradrenaline (b) concentrations versus PaO₂ before (open symbols) and after (black symbols) anaesthesia with MS222 (squares), metomidate (triangles) and AQUI-S (diamonds) or from control fish (circles). The vertical dashed line indicates the P₅₀ of Chinook salmon blood.

DISCUSSION

An "average" SMR for fish was calculated to be $2.78 \pm 1.06 \text{ mM} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$ (1SD) of oxygen (Brett and Groves, 1979). The data for spotties ($1.9 \pm 0.6 \text{ mM} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$ (1SD)) fell within this range of values at the low end. This suggests the fish were relatively unstressed by enclosure within the respirometers.

The respirometry data provided useful information about recovery from anaesthesia. Firstly, it showed that anaesthesia with any of the anaesthetics caused an oxygen deficit at some point during recovery as evidenced by elevated respiration rates compared to the standard metabolic rate. The cause of the deficit is not known but it is most likely due to either a reduced rate of uptake

of oxygen, as suggested by the metomidate data, and/or an increased metabolic rate. A common occurrence in fish undergoing anaesthesia, and one which is used to help determine the depth of anaesthesia, is a reduction in ventilation rate and amplitude (see Bell, 1987; Brown, 1988; Iwama *et al.*, 1989; Ryan, 1992a; Ryan, 1992b; Schoettger and Julin, 1967) which would impair oxygen uptake. A decrease in the rate of oxygen consumption during anaesthesia has been observed in several species of fish using different anaesthetics (goldfish (*Carassius auratus*) with MS222 (Baudin, 1932a), sockeye salmon (*Oncorhynchus nerka*) with MS222 (Blahm, 1961), common blenny (*Blennius pholis*) with quinaldine (Dixon and Milton, 1978), a variety of mullet fry with seven different anaesthetics (Durve, 1975), platyfish (*Xiphophorus maculatus*) with 2-phenoxyethanol, metomidate, MS222 and quinaldine sulphate (Guo *et al.*, 1994; Guo *et al.*, 1995a), killifish (*Fundulus heteroclitus*) with tertiary amyl alcohol, methylparafynol, chlorobutanol and MS222 (McFarland, 1960), guppies (*Poecilia reticulata*) with 2-phenoxyethanol (Teo and Chen, 1993), carp (*Cyprinus carpio*) with 2-phenoxyethanol (Yamamitsu and Itazawa, 1988)). Low concentrations of anaesthetics and other chemicals have been used to decrease the metabolic rate and stress levels in fish in order to increase the efficiency of live fish transport (Barton and Peter, 1982; Davis *et al.*, 1982; Durve, 1975; Erikson *et al.*, 1997; Ferreira *et al.*, 1984b; Guo *et al.*, 1994; Guo *et al.*, 1995b; Hattingh and Van Pletzen, 1974; Martin and Scott, 1959; McDonald *et al.*, 1993; McFarland, 1960; Murai *et al.*, 1979; Patil *et al.*, 1994; Robertson *et al.*, 1988; Rodman, 1963; Ross *et al.*, 1993; Sado, 1985; Takashima *et al.*, 1983; Taylor and Solomon, 1979; Teo and Chen, 1993; Webb, 1958; Wedemeyer *et al.*, 1985). A reduction in the rate of excretion of metabolic products has also been observed (Guo *et al.*, 1995b) which supports the notion that metabolic rate is suppressed. However, with shorter term use of higher concentrations of anaesthetics, as in this study, the most likely effects are a reduced ventilation rate and possibly a subsequent increased metabolic rate and oxygen debt leading to higher rates of oxygen consumption post-anaesthesia.

It is generally recognised that fish have relatively long recovery periods after being anaesthetised with metomidate, and its analogues etomidate and propoxate (Amend *et al.*, 1982; Brown, 1988; Gilderhus and Marking, 1987; Mattson and Riple, 1989). The respiration data supports this to some extent. If it is assumed that at least part of the oxygen debt was caused by a reduced oxygen uptake due to anaesthetic impaired ventilation then these data showed that recovery from impaired gas exchange was faster in the MS222 and Aqui-S treated fish than in the metomidate treated fish. However, contrary to this idea, the rate of recovery from the oxygen debt does not differ between the anaesthetics and occurred within 6 hours. This may have been partially due to, firstly, the difference in variations in recovery time (standard error of metomidate more than twice as high as the standard errors of either MS222 or Aqui-S) which weakens statistical analysis, and secondly, due to a lack of sensitivity of flow through respirometry for finding the exact time that events occurred.

Finally, these data suggest that the oxygen debt caused by the anaesthetics was smaller than that caused by the moderate handling during the setting up of the experiment. This is an important point given that fish anaesthetics are often used to reduced stress in fish during aquaculture practices.

The mean resting heart rate and dorsal aortic pressure of the Chinook salmon used in the Series 2.2 experiments (50.8 ± 3.1 beats per minute (2 SEMs, $n = 63$) and 34.0 ± 1.4 mmHg (2 SEMs, $n = 41$) respectively) are similar to those measured in other salmonids. The resting heart rates and dorsal aortic pressures of rainbow trout used in the study of Fredricks *et al.* (1993) were 49 - 61 beats per minute and 33.8 - 37.9 mmHg respectively at similar temperatures (14 °C in the present study, 12 °C in the study of Fredricks *et al.*, 1993). Resting heart rate and dorsal aortic pressure in brook trout (*Salvelinus fontinalis*) was 36 - 42 beats per minute and 44 - 48 mmHg respectively at 10 °C. Other dorsal aortic blood pressures for resting rainbow trout are 38.8 and 31.2 mmHg (Kiceniuk and Jones, 1977, and Wood and Shelton, 1980, respectively, cited in Burleson *et al.*, 1992). The resting variables in the present study are in the lower range of those recorded in the literature cited which suggests the fish were relatively unstressed considering the invasive nature and confined space of the experimental set up.

The two possible sources of stress in fish anaesthesia are handling prior to anaesthetic induction and the anaesthetic induction itself. Experiment 2.2a was designed to eliminate any handling or disturbance prior to anaesthesia and the data from the control fish suggest this was mostly successful. Control fish showed only very mild, not statistically significant, changes in their cardiovascular variables. Some fish exhibited a transient bradycardia which caused a slight drop in mean heart rate. The fish were most likely reacting to a change in water flow or temperature when the water source was swapped from the normal supply to that from the anaesthetic bucket. The result was probably a startle response. Startle responses occur in fish when they detect almost any change in their environment, such as water disturbance, vibration or a change in lighting. Such responses can result in chronotropic or inotropic modification of the heart (Randall, 1970; Satchell, 1991).

The cardiovascular effects of the anaesthetic exposures varied greatly with the type of anaesthetic. MS222 treated fish showed little change in heart rate, cardiac output or stroke volume. The major effect was a continuous decline in dorsal aortic pressure for the entire exposure to a final value of 75% of the pre-anaesthesia value. This could be caused by reduced resistance downstream (systemic vasodilation), an increased resistance upstream (increased branchial resistance) or a reduction in the force of pumping by the heart (compromised myocardium). MS222 is a local anaesthetic and local anaesthetics affect all types of muscle. Therefore a negative inotropic effect of MS222 on the heart might be expected. If the heart could maintain its current work load no effect

would be noticed, but if its power output decreased sufficiently, stroke volume would decrease leading to a decrease in cardiac output. Cardiac output and stroke volume were unaffected in the MS222 treated fish so a compromised heart is unlikely to be a major contributing factor leading to the reduced dorsal aortic pressure. The possible consequences of direct anaesthetic effects on the heart in the whole animal are discussed in more detail in Chapter 3. Increased branchial resistance would increase the ventral aortic pressure, assuming that the force of contraction of the heart remains constant, but this might also decrease stroke volume as the semilunar valves between the ventricle and *bulbus arteriosus* would remain open for a shorter proportion of the ventricular contraction (Forster, 1989). Therefore, this is also unlikely to be a major contributing factor which leaves a systemic vasodilation as the most likely cause of the observed reduction in blood pressure in the dorsal aorta. Local anaesthetics can cause systemic hypotension in mammals via sympathetic blockade, which can occur during epidural block, or via direct action of the drug on the smooth muscle in the systemic vasculature (Hall and Clarke, 1991). Data on the direct effects of MS222 on blood vessels are presented and discussed in Chapter 4.

Hypotension in mammals is generally caused by blood loss reducing cardiac output due to a reduced venous return to the heart. Severe blood loss leads to shock which is characterised by a rapid heart rate. Another common response to hypotension is vasoconstriction which should restore blood pressure, venous return and cardiac output. The MS222 and Aqui-S treated fish both exhibited a reduction in dorsal aortic pressure. There was no measurable response to this hypotension as vasoconstriction was absent or ineffective and heart rate did not increase. General anaesthetics in humans can affect the medullary vasomotor and respiratory centres of the brain, and impair baroreceptors which would reduce the ability of the cardiovascular system to react to changes in blood volume and blood pressure (Hall and Clarke, 1991). MS222 and Aqui-S anaesthetised fish could be affected similarly.

In the MS222 treated fish the dorsal aortic blood PO₂ decreased about 50% to 46 mmHg. *In vitro*, reduced PO₂ (30 - 50 mmHg) typically causes vasodilation, except in the branchial vessels where it causes vasoconstriction (Olson, 1998b). *In vivo* branchial vasoconstriction should elicit a decrease in dorsal aortic pressure and an increase in ventral aortic pressure, but the hypoxic state will affect other organs and induce neural and/or hormonal changes which could reverse the vasoconstriction or obscure its effects. Hypoxia (water PO₂ of 25 - 45 mmHg) in the lingcod (*Ophiodon elongatus*) caused a decrease in dorsal aortic pressure, did not change ventral aortic pressure and caused a decrease in cardiac output (Farrell, 1982) whereas in the Atlantic cod (*Gadus morhua*) hypoxia caused an increase in both ventral and dorsal aortic pressures and decreased the heart rate (Fritsche and Nilsson, 1990). These two studies are not entirely comparable because the former used a slower graded hypoxia, induced over a few minutes,

whereas the later induced hypoxia in less than one minute. Some fish anaesthetics could affect oxygen receptors and/or baroreceptors, as some mammalian general anaesthetics affect baroreceptors (Hall and Clarke, 1991), and anaesthetised fish may be unable to react to hypoxia.

Metomidate had little effect on the cardiovascular variables measured and there were no statistically significant changes. An initial mild bradycardia causing a proportionally similar decrease in cardiac output (both not statistically significant) were very similar to the changes seen in the control fish suggesting this effect was not caused by metomidate. During induction the dorsal aortic pressure was not as stable as pre-induction and the variation increased suggesting it may have been mildly affected. A longer duration of exposure or exposure to a higher concentration of metomidate may have caused a greater change. Etomidate, an analogue of metomidate, is used as an intravenous hypnotic and, unlike many other hypnotics and anaesthetics, its effects on the cardiovascular system of dogs (Reneman and Janssen, 1977), cats (Fischer and Marquort, 1977) and humans is small. The minimal effect of metomidate on fish in this study agrees with these previous findings. Etomidate does, however, reduce coronary and systemic resistance in humans which increases the blood flow to the heart and periphery (Dundee and Clarke, 1979) and causes a slight fall in arterial pressure (Dundee and Clarke, 1979; Reneman and Janssen, 1977). Dorsal aortic pressure did not change significantly in response to metomidate in this study although the mean showed greater variability than pre-anaesthesia. The dorsal aortic blood PO_2 of metomidate treated fish decreased by 59% to 35 mmHg which is low enough to cause vasodilation of blood vessels (Olson, 1998b). This might also have caused some of the increased variation in dorsal aortic pressure.

Aqui-S caused the most impressive response of the three anaesthetics. After a transient response similar to that seen in the control fish heart rate, cardiac output, dorsal aortic pressure and stroke volume all decreased significantly. After reaching minima at between one and 1.5 minutes heart rate, dorsal aortic pressure and stroke volume began to increase again. If the decreases resulted from a direct effect of the anaesthetic then they may be expected to progressively decrease for the entire exposure or at least plateau due to the continuing presence of the anaesthetic. They did not do this, which suggests the decreases were caused by a reaction to the anaesthetic rather than resulting from a direct effect of the anaesthetic. Nociceptors are receptors situated on gills which respond to toxins in the water (Satchell, 1991). Common nociceptor mediated responses are bradycardia, hypotension, shallow respiration and a brief cessation of swimming. The significance of these actions is to firstly, decrease the amount of toxin uptake by reducing flow over the gills via reduction of movement and ventilation, and secondly, to interrupt transport of the toxin from gills to tissues via bradycardia which would cause hypotension (Satchell, 1991). The fish exposed to Aqui-S showed some of these responses. Cardiac output was definitely reduced both by a

reduction of heart rate and of stroke volume. Also, as a result, dorsal aortic pressure decreased. However, activity increased a large amount. The fish were housed in boxes and could not escape the inflowing anaesthetic solution. The suggested reason for cessation of movement in the nociceptor response is escape from the toxin. In this experiment frantic movement was the only available option fish had of avoiding the anaesthetic. Ventilation was not monitored so cannot be commented on. Isoeugenol is the active component of AQUI-S (Alastair Jerrett pers. comm.) and makes up 70 - 90 % of clove oil (Keene *et al.*, 1998). It is well known that cloves are "hot" when bitten and AQUI-S also has this "hot" sensation when applied to the tongue and lips (pers. obs). This chemical, even though it is greatly diluted, may irritate the gills and eyes of fish and cause the observed response. Rainbow trout exposed to 17 ppm AQUI-S showed an increase in cortisol and haematocrit after a 30 minute exposure. The fish also showed a secondary cortisol increase which was suggested to be indicative of persistent irritation (Dr Glen Davidson, pers. comm).

At between 1.5 and two minutes heart rate, cardiac output and stroke volume began to increase to baseline levels, with cardiac output and stroke volume rising above baseline levels at between 3.5 and four minutes. The initial decrease in dorsal aortic pressure was maintained. The frantic activity of the fish upon exposure to AQUI-S was quite likely to have been anaerobic. Therefore, the increases in heart rate, stroke volume and cardiac output may have been an attempt to recover oxygen debt. This idea is supported by the fact that the PO_2 of the dorsal aortic blood was very low at the end of the exposure period (11% of pre-anaesthesia levels), and significantly lower than the blood PO_2 of the MS222 treated fish at the equivalent time. The positive cardiac inotropy and chronotropy must have been mediated humorally and/or neurally. The dorsal aortic blood taken post-induction contained more adrenaline and noradrenaline than the pre-induction blood which shows the changes in the heart could have been mediated humorally. However, the plasma catecholamine increase was relatively small compared to stress responses from other stressors (Randall and Perry, 1992). No data were collected regarding sympathetic neural activity. Haematocrit increased, which suggests there was sufficient neural and/or humoral stimulation to cause a splenic response. Dorsal aortic pressure remained low even though the other three variables returned to baseline levels. The cause of this maintenance of hypotension may be analogous to the decrease in dorsal aortic pressure observed in the MS222 treated fish. Similarities and differences between the vasoactivity of the three anaesthetics may help explain this. The effect of the three anaesthetics on vasoactivity will be examined in Chapter 4. As most AQUI-S treated fish became quite active for a short period of time at the beginning of the exposure, cardiovascular changes due to exercise may have occurred. Moderate exercise causes an increase in cardiac output via increases in heart rate and stroke volume (Satchell, 1991). An increase in these three variables was not observed until one to 1.5 minutes into the induction and did not reach pre-

exposure levels until at least 2.5 minutes which suggests exercise had little or no effect on the outcome.

The mean dorsal aortic blood PO₂ decreased significantly in all of the anaesthetic treated fish. The most likely reason for this is a reduction in the rate and amplitude of ventilation. Due to the relatively low oxygen content of water a large volume must pass over the gills of fish in order for them to get enough oxygen. Fish with a reduced ventilation volume would soon exhibit hypoxic blood.

The mean baseline concentrations of plasma adrenaline and noradrenaline from all fish combined were 2.19 ± 0.14 and 0.33 ± 0.08 nmol.L⁻¹ respectively. The concentrations of adrenaline and noradrenaline in unanaesthetized cannulated fish is usually between one and five nmol.L⁻¹ (Randall and Perry, 1992) although lower levels have been reported (Thomas and Perry, 1992). Some of the baseline noradrenaline concentrations in this study were below the detection levels of the technique and were considered to be zero. The detection limit of the EC-HPLC technique was about 0.1 nmol.L⁻¹ for both catecholamines.

The plasma catecholamines of the Aquí-S treated fish increased post-exposure. A dominant factor controlling catecholamine release is low blood oxygen content and the principal effect of the release of catecholamines in this case is enhancement of blood oxygen transport (Randall and Perry, 1992). It has been suggested that chromaffin tissue can release catecholamines due to the direct effect of local hypoxaemia (Randall and Perry, 1992) and that the critical blood oxygen content required for this effect to occur is about 50 - 60 % of saturated levels (Randall and Perry, 1992; Thomas and Perry, 1992). For this reason the haemoglobin oxygen equilibrium curves were constructed. The P₅₀ of Chinook salmon blood was calculated to be 26.3 ± 1.9 torr, which is close to the values reported for rainbow trout (*Oncorhynchus mykiss*) at comparable temperatures. Nikinmaa and Soivio (1979) report a P₅₀ of 21.6 mmHg at 12 °C, Soivio *et al.* (1980) report a value of 21.0 at 11 °C and Tetens and Christensen (1987) give a P₅₀ of 23.2 at 15 °C. All Aquí-S treated fish that contained elevated plasma adrenaline or noradrenaline concentrations (> five nmol.L⁻¹)² had a PaO₂ of less than 26 torr. However, only half (four of eight) of those with a blood PO₂ of less than 26 torr exhibited elevated plasma catecholamines. In addition, two metomidate treated fish also had a PaO₂ of 26 torr or less and did not show elevated plasma catecholamines (see Figures 2.10a&b). This suggests a PaO₂ below the P₅₀ could have caused the elevated plasma catecholamines but there is obviously variation between individuals. This variation was also seen in the data of Thomas and Perry (1992) and Perry and Reid (unpublished data, cited in Randall and

² A plasma catecholamine concentration of 5 nmol.L⁻¹ was selected as being an elevated level from Figures 2.10a&b

Perry, 1992) as in these studies there were a significant number of fish that were sufficiently hypoxaemic but had not released catecholamines into their blood. Plasma catecholamine levels also increase after a variety of other physical and environmental disturbances such as hypercapnia, air exposure, exhaustive or “violent” exercise, metabolic acidosis, anaemia and exposure to “soft water” (see Randall and Perry, 1992). Some of these events do not necessarily coincide with hypoxia. Also, catecholamines can be released from sources other than the chromaffin tissue. Catecholamines can be released as overflow from adrenergic nerves (Esler *et al.*, 1990; Randall and Perry, 1992). The effects of this are probably local, as the total amount of catecholamine released is small, but it is conceivable that this source could increase circulating catecholamines slightly (Randall and Perry, 1992). The spleen and heart are possible sites of neural catecholamine overflow.

Another consideration is when the catecholamines were released. Hypoxia would be greatest late in the anaesthetic exposure which suggests that if the release was due to hypoxia then the catecholamines would also be released late in the exposure. Therefore, catecholamine concentrations may have been relatively low because the blood PO₂ had only just reached a level where catecholamines are released. The activity of the Aquil-S treated fish would have caused blood PO₂ to decrease more quickly, due to an increased metabolic rate, which would have allowed these fish to reach the critical blood PO₂ prior to the fish treated with MS222 and metomidate. A longer exposure to any of the anaesthetics, causing a more prolonged hypoxia, may have caused a greater increase in catecholamines.

If catecholamines were released early in the exposure they may have been removed from the plasma during the time between release and blood sampling. Gills can quickly sequester or metabolise catecholamines (Olson, 1998a). Isolated rainbow trout gills were found to remove 60% and 47% of a single pulse of noradrenaline and adrenaline respectively, and 7% and 30% of noradrenaline and adrenaline, respectively, if applied continuously (Nekvasil and Olson, 1986a). Other tissues such as kidney, spleen, liver and atrium also sequester and/or metabolise catecholamines (Nekvasil and Olson, 1986b). In the whole trout (*Oncorhynchus mykiss*) 50% or more of catecholamines are removed from the circulation in ten minutes and of the remaining over 80% were metabolised to inactive forms within that time (Nekvasil and Olson, 1986b).

The fish used in this experiment were almost certainly chronically stressed due to the anaesthesia and surgery and enclosure in a confined box. This could affect future stress reactions via down regulation of catecholamine production or desensitisation and/or sensitisation to stressors (Mutschler and Derendorf, 1995). A single blood sample from a fish immediately post-anaesthesia and surgery (prepared for Experiment 2.2a) had high plasma adrenaline and noradrenaline levels

(251.4 and 48.8 nmol.L⁻¹ respectively) compared to the Aqui-S post-anaesthesia levels. However, the stress of surgery was almost certainly greater than the stress caused by Aqui-S anaesthesia alone which could account for the large difference. Catecholamine release by rainbow trout (*Oncorhynchus mykiss*) after anaesthesia and surgery has been measured previously with methods very similar to the present study (Gingerich and Drottar, 1989). In their studies, after the surgery, which consisted of dorsal aortic cannulation, plasma adrenaline and noradrenaline were measured at about 80 and 30 nmol.L⁻¹ respectively. However, after a 48 hour recovery period and subsequent MS222 re-anaesthesia adrenaline and noradrenaline rose to only about 3.7 and 2.3 nmol.L⁻¹. These increases are very similar to those measured in the present study.

Aqui-S treated fish also showed an increase (11.5%) in haematocrit. Haematocrit can increase due to recruitment of erythrocytes from the spleen and/or erythrocyte swelling. The spleen expels erythrocytes at low plasma catecholamine levels via the action of α -adrenoceptors (see Randall and Perry, 1992). Erythrocyte swelling occurs as a result of proton and sodium ion exchange across the red blood cell membrane creating a higher concentration of sodium in the cell. Water passively follows and causes the cell to swell. The removal of protons from the erythrocytes increases the pH in the cell allowing haemoglobin to bind oxygen more efficiently. The increase in haematocrit in the Aqui-S treated fish was consistent with, and was most likely caused by, the observed increase in catecholamines. Both of these events probably occurred late in the exposure due to moderate to severe hypoxaemia.

Experiment 2.2b showed the effect of stress prior to anaesthesia on heart rate, cardiac output and stroke volume. Although there were statistically significant differences between the anaesthetics, at least for cardiac output and heart rate, the trends shown by the different anaesthetics were similar which suggests that handling had a greater effect than any of the anaesthetics. On initiation of recording, cardiac output was elevated due to an increase in stroke volume and probably heart rate, although the heart rate increases for metomidate and Aqui-S were not statistically significant. The likely cause of this is neural and/or humoral adrenergic stimulation. Blood samples were not taken in this experiment so an increase in plasma catecholamines was not verified. Eventually heart rate and cardiac output began to decrease, possibly as adrenergic stimulation declined. Heart rate dropped to below pre-anaesthesia levels in the metomidate and Aqui-S treated fish. A similar decrease in the MS222 treated fish may have been obscured by the higher variation. This decrease cannot be explained by the removal of adrenergic stimulation. A partial explanation may come from the fact that the rate of decrease of heart rate was fastest for metomidate. This suggests two possibilities: 1) that, as the only difference between the treatments was the anaesthetics used, metomidate caused a faster decline in heart rate. As some of the other data collected (Series 2.1) suggests ten ppm metomidate is a disproportionately high concentration of anaesthetic compared

to 100 ppm MS222 and 60 ppm Aqui-S, then this suggests that 2) MS222 and Aqui-S could also affect heart rate. Lochowitz *et al.* (1974) showed that increased vagal tone reduced the heart rate in MS222 treated fish. This could be the cause in Experiment 2.2b. This decrease in heart rate was not observed in Experiment 2.2a possibly due to the shorter duration in that experiment. The electrocardiograph of carp (*Cyprinus carpio*) exposed to 800 ppm 2-phenoxyethanol for two hours showed an increase in QT interval whereas two hours exposure to 400 ppm 2-phenoxyethanol caused a decrease in QT intervals (Yamamitsu and Itazawa, 1988). All the carp exposed to 800 ppm 2-phenoxyethanol stopped respiring and died, probably from hypoxia, whereas the 400ppm exposed fish did not stop ventilating and could be revived. This suggests that heart rate was reduced via an effect of hypoxaemia which may also be the case in Experiment 2.2b.

The difference between anaesthesia with and without prior handling is large. The cardiovascular changes due to MS222 and metomidate anaesthesia are minor compared to those caused by handling. Qualitatively the differences between Aqui-S anaesthesia with and without handling are great but the magnitude of the changes are similar. Because of the qualitative differences it is difficult to say whether Aqui-S anaesthesia alone has less of an effect than with handling.

The cardiovascular effects of anaesthetics have been studied in fish a number of times. The measurement of different variables, the use of different anaesthetics and different concentrations of anaesthetic, and also the use of different methods of anaesthesia make a comparison of different publications difficult. Heart rate has been measured in a number of fish species undergoing MS222 anaesthesia. The results of these studies are summarised in Table 2.2. Upon initial exposure, fish can exhibit an increase, decrease or no change in heart rate. Initial tachycardias are quite common and may be caused by a number of factors. It has been suggested that an initial tachycardia occurs as a result of “central depression of tonic cardiovagal inhibition” (Houston *et al.*, 1971a). It could also be due to a mild startle response caused by the delivery of anaesthetic, as extreme measures must be taken to avoid disturbance of fish during anaesthesia, or by over-sensitivity of the fish, as some fish species seem to be more sensitive to anaesthetics than others (Randall, 1962; Randall and Smith, 1967).

No change in heart rate was observed on some occasions (Table 2.2). This may be a real effect or it could be due to the size of the change being smaller than the variability. The initial decrease in heart rate seen in elasmobranchs (Campbell and Davies, 1963) is probably due to the method of application and the concentration used (1700 ppm). The anaesthetic solution was applied to the pharyngeal cavity, which must have involved moderate handling, and the solution was probably quite acidic so the bradycardia maybe a nociceptor type response. As the concentration is so high, deep anaesthesia could have been reached very quickly as well.

Table 2.2. Heart rates of fish following MS222 exposure. See text for explanation.

Species	ppm	Buffered	Stressful	Initial	Progressive	Reference
rainbow trout (<i>Oncorhynchus mykiss</i>)	67	SW	no	NC	decr	(Randall <i>et al.</i> , 1965)
rainbow trout (<i>O. mykiss</i>)	75-150	no?	no	NC	decr	(Randall and Smith, 1967)
brook trout (<i>Salvelinus fontinalis</i>)	100	no?	no	tachy	decr	(Houston <i>et al.</i> , 1971a)
carp (<i>Cyprinus carpio</i>)	100	?	?	tachy	decr	(Serfaty <i>et al.</i> , 1959)
Chinook salmon (<i>O. tshawytscha</i>)	100	yes	yes	tachy	decr	present study
rainbow trout (<i>O. mykiss</i>)	40-100	no?	no	tachy	decr	(Lochowitz <i>et al.</i> , 1974)
Chinook salmon (<i>O. tshawytscha</i>)	100	yes	no	NC	NC	present study
dogfish (<i>Squalus acanthias</i>)	50	SW	no	?	NC	(Peirce and Peirce, 1967)
rainbow trout (<i>O.s mykiss</i>)	51	yes	no	NC	NC	(Fredricks <i>et al.</i> , 1993)
tench (<i>Tinca tinca</i>)	33	no?	no	tachy	incr	(Randall, 1962)
elasmobranchs	1700	no?	yes	brady	decr	(Campbell and Davies, 1963)

ppm = concentration of MS222
SW = buffered with seawater
? = unknown
tachy/brady = tachycardia/bradycardia
decr/incr = decrease/increase
NC = no change

As MS222 anaesthesia progresses heart rate can again increase, decrease or remain static (Table 2.2). The increase and maintenance of heart rate are almost certainly due to low concentrations of anaesthetic whereas higher concentrations will always cause a decrease. It has been suggested this decrease is caused by an increase in vagal tone in response to hypoxaemia (Lochowitz *et al.*, 1974). Lochowitz *et al.* (1974) could reverse the bradycardia using atropine if it was applied early enough. Late in the exposure atropine could not reverse the bradycardia and it was suggested this was because of a direct effect of MS222 on the myocardium. Another possibility was that it was an effect of prolonged hypoxaemia on the heart. Other anaesthetics may cause different reactions, such as the unusual reaction caused by Aquil-S in this study. Regardless of the anaesthetic, if the concentration is high enough to impair respiration, hypoxaemia will result and lead to a bradycardia unless the vagal tone is blocked.

The effect of fish anaesthetics on cardiac output and stroke volume in the whole fish has, to the knowledge of this author, only been measured once before. The effect of MS222 on dogfish (*Squalus acanthias*) was to decrease cardiac output at all concentrations (Peirce and Peirce, 1967). MS222 at 20 ppm took one hour to cause the decrease but 50 ppm took only five minutes. The decrease was progressive and was almost entirely caused by decreases in stroke volume. In the present study cardiac output did not change in response to MS222 but the exposure time was much smaller. The cardiac outputs from the anaesthesia and stress treatments did fall, but they were initially elevated due to stress, which makes interpretation of a decrease due to anaesthesia difficult. A decrease in cardiac output with anaesthesia may be partially caused by the reduction in locomotion and respiration. These two activities use muscles which compress vessels. This

pumps blood, which moves in one direction because of valves in the vasculature. Examples are the branchial and haemal arch pumps (Satchell, 1991). The value of these pumps is not in supplementing arterial flow but in promoting venous return to the heart. As venous return is an important modulator of stroke volume and hence cardiac output the cessation of locomotion and respiration may lower cardiac output.

MS222 decreases dorsal aortic pressure in fish (present study, Fredricks *et al.*, 1993; Peirce and Peirce, 1967; Randall *et al.*, 1965). These studies looked at different concentrations of MS222 in different fish species which suggests dorsal aortic pressure decrease is a general phenomenon of MS222 anaesthesia. The study of Fredricks *et al.* (1993) is the only work to have measured dorsal and ventral aortic pressure simultaneously and the only other study to have measured the effects of anaesthetics other than MS222 on blood pressure. In that study benzocaine hydrochloride did not effect dorsal or ventral aortic pressure, MS222 and etomidate reduced dorsal aortic pressure, and 2-phenoxyethanol caused a decrease in dorsal aortic pressure and a progressive increase in ventral aortic pressure. The absence of a decrease in ventral aortic pressure indicates that the contractile force of the heart and the branchial resistance did not change significantly (or that they cancel each other out). If they do not change then any concurrent decrease in dorsal aortic pressure is probably due to systemic vasodilation. Higher concentrations of anaesthetic, especially local anaesthetics, may cause cardiac contractility to decline considerably. The normal vasoactivity in fish is controlled by neural, hormonal and local effects (Nilsson, 1994). Normally, vessels in the systemic vasculature have a slight tension caused by a continuous adrenergic tonus induced either neurally and/or humorally. If this tonus is blocked, either by α -adrenoceptor or ganglionic blockade, the vessels relax and a reduction in dorsal aortic pressure is observed (Xu and Olson, 1993). Mammalian general anaesthetics such as halothane affect the central nervous system which may decrease the neurally-mediated tonus and cause vasodilation and hypotension (Black, 1980). Fish anaesthetics are generally assumed to have similar effects. Local anaesthetics such as MS222 may block nerves directly (Späth and Schweickert, 1977). In either case these are possible routes for anaesthetics to cause a decrease in dorsal aortic pressure. Another possibility is a direct vasodilatory action of the anaesthetics on the vascular smooth muscle. This possibility is discussed fully in Chapter 3.

The only blood changes in the MS222 and metomidate treated fish were decreases in dorsal aortic blood PO₂. This was almost certainly caused by a reduction in ventilation. If the exposure had continued for longer and the hypoxia increased in severity, more haematological changes may have occurred. The Aquic-S treated fish showed a greater decrease in blood PO₂ than the MS222 treated fish and also an increase in plasma adrenaline and noradrenaline and haematocrit. The latter three changes may well have been solely due to the hypoxia. A number of previous studies

have looked at some or all of these effects. The findings of these studies are summarised in Table 2.3. Although the PO₂ data are limited it does confirm oxygen uptake is impaired during anaesthesia. Studies are divided over whether or not anaesthesia causes an increase in catecholamines and haematocrit. This strongly suggests that factors other than the anaesthetic may be causing the increases in these variables when they occur. These factors are likely to include the method of anaesthetisation (how much handling is involved), interspecific differences (flatfish seem to be highly resistant to anaesthesia, see Bourne, 1984; Malmstrom *et al.*, 1993), secondary properties of the anaesthetics (acidity of MS222 and benzocaine hydrochloride, the irritant properties of Aqui-S) and the depth and duration of anaesthesia.

Recovery of cardiovascular variables after exposure to any of the anaesthetics occurs rapidly. In the MS222 and metomidate treated fish there was a slight increase in heart rate (not statistically significant) and dorsal aortic pressure (statistically significant) for about five to ten minutes post-anaesthesia. These changes are mild and may be caused by the sensation of coming out of anaesthesia or the recovery from oxygen debt and hypoxia. An increase in heart rate and an increase in blood pressure suggest a recovery of sympathetic tonus. Very similar effects were seen in rainbow trout (*Oncorhynchus mykiss*) recovering from dorsal aortic cannulation (Randall *et al.*, 1965) and hypoxia (Randall and Smith, 1967). Cardiac output was fairly constant except for an increase at six hours. The reason for this is not clear. Stroke volume was constant. The recovery of the Aqui-S treated fish was quite similar except for a small, variable increase in stroke volume which lasted for about one hour. It was only significantly higher than control values at 30 minutes. The increase in stroke volume was mirrored by an increase in cardiac output (not statistically significant). The reasons for this are unclear.

MS222 heart rate recovery data of fish from the study of Fredricks *et al.* (1993) are similar to the present study with no deviation from pre-anaesthesia values. Etomidate and 2-phenoxyethanol treated fish showed a large decrease in heart rate during anaesthesia which took up to 30 minutes of recovery to reach pre-anaesthesia values. Benzocaine hydrochloride treated fish showed an increase in heart rate for up to two hours post-anaesthesia. A possible reason for this was not offered but as dorsal and ventral aortic pressure were elevated for similar periods of time, catecholamine release or a neural sympathetic response may have been the cause.

During recovery from anaesthesia and surgery there were large deviations in the measured cardiovascular variables for up to 24 hours. These deviations were likely to be due to an additive effect of handling and anaesthesia causing hypoxia and stress. The differences between anaesthetics were minor although there was a statistically significant interaction between anaesthetic used and time for the heart rate data. Initially there was a small decrease in heart rate

Table 2.3. Dorsal aortic PO₂, haematocrit and plasma catecholamine changes in various species of fish after exposure to anaesthetics.

[anaesthetic]	Species	time	PO ₂	HCT	cats	Reference
MS222						
60-100 ppm	<i>Pagothenia borchgrevinkii</i>	7 - 8 min		incr		(Ryan, 1992a)
70 ppm	crucian carp (<i>Carassius carassius</i>)	imob		incr		(Hoffmann <i>et al.</i> , 1982)
70 ppm	rainbow trout (<i>Oncorhynchus mykiss</i>)	imob		incr		(Hoffmann <i>et al.</i> , 1982)
70 ppm	rainbow trout (<i>O. mykiss</i>)	3 - 9 min		incr		(Reinitz and Rix, 1977)
<100 ppm	snapper (<i>Pagrus auratus</i>)	< 5 min		NC		(Ryan, 1992b)
100 ppm	snapper (<i>P. auratus</i>)	5 min		incr		(Ryan, 1992b)
100 ppm	rainbow trout (<i>O. mykiss</i>)	deep	decr	NC?	incr	(Iwama <i>et al.</i> , 1989)
100 ppm	rainbow trout (<i>O. mykiss</i>)	15 min	decr	NC		(Soivio <i>et al.</i> , 1977)
100 ppm	rainbow trout (<i>O. mykiss</i>)	15 min		incr		(Houston <i>et al.</i> , 1971b)
100 ppm	rainbow trout (<i>O. mykiss</i>)	15 min		incr		(Houston <i>et al.</i> , 1971a)
100 ppm	rainbow trout (<i>O. mykiss</i>)	deep			incr	(Gingerich and Drottar, 1989)
100 ppm ³	rainbow trout (<i>O. mykiss</i>)	deep		NC?		(Smit <i>et al.</i> , 1979a)
100 ppm	plaice (<i>Pleuronectes platessa</i>)	20 min ¹		NC		(Bourne, 1984)
100 ppm	dab (<i>Limanda limanda</i>)	20 min ¹		NC		(Bourne, 1984)
100 ppm	turbot (<i>Scophthalmus maximus</i>)	20 min ¹		NC		(Bourne, 1984)
100 ppm ³	carp (<i>Cyprinus carpio</i>)	deep		incr?		(Smit <i>et al.</i> , 1979a)
100 ppm ³	<i>Sarotherodon mossambicus</i>	deep		incr?		(Smit <i>et al.</i> , 1979a)
100 ppm	splake	15 min		incr		(Nieminen <i>et al.</i> , 1982)
240 ppm	halibut (<i>Hypoglossus hypoglossus</i>)	deep		NC		(Malmstrom <i>et al.</i> , 1993)
500 ppm	blacknose dace	deep		NC		(MacAvoy and Zaepfel, 1997)
? ppm ²	carp (<i>C. carpio</i>)	imob		incr	incr	(Jeney <i>et al.</i> , 1986)
100 ppm buffered	Chinook salmon (<i>O. tshawytscha</i>)	5 min	decr	NC	NC	present study
100 ppm buffered	rainbow trout (<i>O. mykiss</i>)	15 min		incr		(Soivio <i>et al.</i> , 1977)
100 ppm buffered ³	carp (<i>C. carpio</i>)	deep		incr?		(Smit <i>et al.</i> , 1979a)
100 ppm buffered ³	rainbow trout (<i>O. mykiss</i>)	deep		NC?		(Smit <i>et al.</i> , 1979a)
100 ppm buffered ³	<i>S. mossambicus</i>	deep		NC?		(Smit <i>et al.</i> , 1979a)
300 ppm buffered	pike (<i>Esox lucius</i>)	deep		NC		(Oikari and Soivio, 1975)
300 ppm buffered	sculpin (<i>Myoxocephalus scorpius</i>)	deep		NC		(Oikari and Soivio, 1975)
300 ppm buffered	sculpin (<i>M. quadricornis</i>)	deep		NC		(Oikari and Soivio, 1975)
300 ppm buffered	blenny (<i>Zoarces viviparus</i>)	deep		NC		(Oikari and Soivio, 1975)
benzocaine						
30 ppm	rainbow trout (<i>O. mykiss</i>)	deep	decr	incr	incr	(Iwama <i>et al.</i> , 1989)
33 ppm	rainbow trout (<i>O. mykiss</i>)	15 min		incr		(Soivio <i>et al.</i> , 1977)
benzocaine HCl						
?? ppm	carp (<i>C. carpio</i>)	deep		incr?		(Ferreira <i>et al.</i> , 1981)
?? ppm buffered	carp (<i>C. carpio</i>)	deep		NC?		(Ferreira <i>et al.</i> , 1981)
metomidate						
2-10 ppm	Atlantic salmon (<i>Salmo salar</i>)	60 min		incr		(Olsen <i>et al.</i> , 1995)
2-10 ppm	Atlantic salmon (<i>S. salar</i>)	30 min		NC		(Olsen <i>et al.</i> , 1995)
5 ppm	rainbow trout (<i>O. mykiss</i>)	deep	decr	NC?	incr	(Iwama <i>et al.</i> , 1989)
7 ppm	Chinook salmon (<i>O. tshawytscha</i>)	5 min	decr	NC	NC	present study
30 ppm	halibut (<i>Hypoglossus hypoglossus</i>)	deep		NC		(Malmstrom <i>et al.</i> , 1993)
etomidate						
2 ppm	channel catfish (<i>Ictalurus punctatus</i>)	30 min		incr		(Limsuwan <i>et al.</i> , 1983b)
Aqui-S						
60 ppm	Chinook salmon (<i>O. tshawytscha</i>)	5 min	decr	NC	NC	present study
Propanidid						
2068 ppm ²	carp (<i>C. carpio</i>)	imob		incr	incr	(Jeney <i>et al.</i> , 1986)
2-phenoxyethanol						
200 ppm	rainbow trout (<i>O. mykiss</i>)	deep	decr	NC?	incr	(Iwama <i>et al.</i> , 1989)
CO₂						
50 % in air	rainbow trout (<i>O. mykiss</i>)	deep	NC	NC?	incr	(Iwama <i>et al.</i> , 1989)
ethyl carbamate						
3 %	eel (<i>Anguilla anguilla</i>)	10 min			NC	(Le Bras, 1982)
TCB						
900 ppm	crucian carp (<i>C. carassius</i>)	imob		NC		(Hoffmann <i>et al.</i> , 1982)
900 ppm	rainbow trout (<i>O. mykiss</i>)	imob		NC		(Hoffmann <i>et al.</i> , 1982)

cats = catecholamines, imob = immobilised, TCB = trichlor-butanol, NC = no changes, incr/decr = increase/decrease, deep = deep anaesthesia

¹ fish not deeply anaesthetised

² also injected with hormone

³ 25-150 ppm were used

and a large increase in stroke volume which caused an overall increase in cardiac output. This lasted for about one hour. The increase in stroke volume suggested an adrenergic inotropic stimulation. However, this should be accompanied by an increase in heart rate and was not. The decrease in heart rate suggests increased vagal tonus. The cause of these effects might be discovered using adrenergic and acetylcholine blockers. Heart rate then increases to above pre-anaesthesia values and stroke volume returns to normal resulting in a slightly increased cardiac output. This effect lasted for up to six hours. Over the last 12 hours of recovery heart rate, stroke volume and cardiac output were not different from the control. Recovery of brook trout (*Salvelinus fontinalis*) and carp (*Cyprinus carpio*) from anaesthesia and surgery has been examined previously (Houston *et al.*, 1973). The heart rate of trout remained elevated for at least 26 hours (data collection ended at 26 hours) whereas the heart rate of carp progressively decreased for 12 hours to about 50% of the original values and maintained this until the end of the experiment. These results are very different from those presented in this study.

Recovery from severe physical stress seems to be characterised by a reduced heart rate and an increased cardiac output due to an elevated stroke volume for up to two hours. This type of effect was seen to a lesser degree in the recovery Aqui-S treated fish not subjected to handling. This observation along with the increase in catecholamines, increase in haematocrit, and struggling during anaesthetic induction strongly suggests Aqui-S at the concentration tested causes fish some physical distress.

Chapter 3

Acute effects of MS222, metomidate and Aqui-S on teleost heart function

INTRODUCTION

Impaired cardiac function during anaesthesia could potentially have detrimental consequences. For example, impaired gas transport could lead to hypercapnia and hypoxia or anoxia which would lead to death. A reduced cardiac output could also increase the recovery time. Some fish anaesthetics are removed from the site of action rather than being metabolised to an inert form. For example, in dogfish (*Squalus acanthias*) 95% of accumulated MS222 is excreted by the gill in the first two hours of recovery whereas only 5% is metabolised and excreted via the kidney (Stenger and Maren, 1974). This suggests adequate blood flow is required for recovery from anaesthesia.

Cardiac output is a product of heart rate and stroke volume. Heart rate and stroke volume can be manipulated neurally, hormonally and physically, increasing or decreasing cardiac output in an effort to meet demand. If an exogenous agent affected heart rate or stroke volume and/or interfered with the intrinsic cardiovascular controls, cardiac output could be compromised. Skeletal muscle contractions also help move blood, especially through the venous system where blood pressures are low (Satchell, 1991). As anaesthetised fish are often immobilised, greater emphasis is placed on the heart for an adequate blood supply.

Local anaesthetics affect nerves and muscles by interfering with ion movements. Nerves become incapable of transmitting action potentials and all types of muscle lose contractile ability and release tension (Rowland, 1974). In mammals, local anaesthetics can cause the heart to reduce contractile force and induce arrhythmias (Hall and Clarke, 1991). Neural control of the heart may also be impaired. Some general anaesthetics such as halothane, enflurane, isoflurane and nitrous oxide are known to reduce the contractile ability of the heart *in vitro* but often no effects are seen *in vivo*, probably due to compensatory homeostatic mechanisms (Stoelting and Miller, 1989).

MS222, a chemical with local anaesthetic properties, is known to affect the contractility of the heart of rainbow trout (Ryan *et al.*, 1993). Similarly, analogues of the active component of Aqui-S significantly affect the cardiovascular systems of mammals (Dundee and Clarke, 1979). The first

experiment in this chapter aims to corroborate the effects of MS222 on fish hearts shown by Ryan *et al.* (1993) using a different fish species, and also to compare the effects of MS222 with the effects of metomidate and Aqui-S, which have different mechanisms of action. Strips of ventricle from Chinook salmon (*Oncorhynchus tshawytscha*) were stimulated to contract and the force of the contractions measured. The strips were exposed to the anaesthetics under investigation and the changes the anaesthetics caused were monitored.

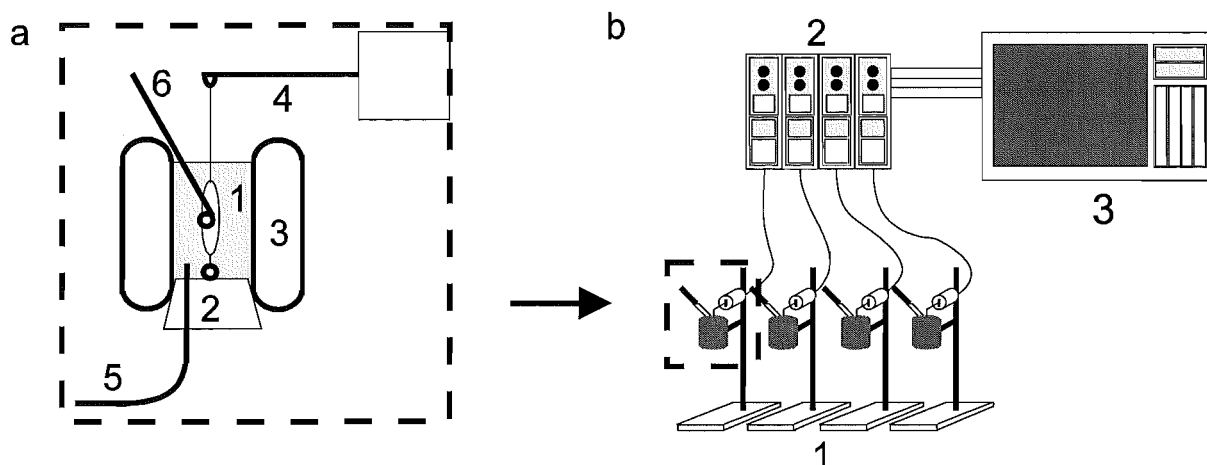
Vagal outflow can decrease the heart rate in teleosts and elasmobranchs (Olson, 1998b; Randall, 1970; Satchell, 1991) and, in some teleosts, can increase the heart rate via a sympathetic branch (Farrell, 1992; Saito and Tenma, 1976; Satchell, 1991; Taylor, 1992). It has been suggested that MS222 can block excitatory adrenergic vagal stimulation of the heart of *Girella tricuspidata* (Montgomery *et al.*, 1986). By contrast, a decrease in heart rate of *Oncorhynchus mykiss* after 30 minutes of 100 ppm MS222 anaesthesia was attributed to maximum cholinergic vagal inhibition and was reversed by intraperitoneal and/or intrapericardial injections of atropine (Lochowicz *et al.*, 1974). Other authors suggest tonic cardiovagal inhibition is depressed centrally during MS222 anaesthesia (Houston *et al.*, 1971a). The second experiment in this chapter examines the effects of MS222, metomidate and Aqui-S on the cardiac branch of the vagus and its ability to regulate a spontaneously beating perfused heart preparation. Anaesthetic was applied to the stimulated vagus nerve to see if the anaesthetics could alter conduction down the nerve.

MATERIALS AND METHODS

SERIES 3.1. The effects of MS222, metomidate and Aqui-S on paced ventricle strips.

Diagrams of the experimental set up for Series 3.1 are shown in Figures 3.1a&b. Chinook salmon (mass 78 ± 8 g (2 SEMs), range 58.0 - 117.0 g, $n = 16$) were killed with a blow to the head. The heart was exposed, excised and placed in a 15 °C fresh water fish Ringer's solution¹ in a Petri dish. The Ringer's solution had been bubbled with a 0.5% CO₂ in air gas mixture. The atrium was removed and the *bulbus arteriosus* was trimmed to within two to three mm of the ventricle (ventricle mass 55 ± 7 mg (2 SEMs), range 34 - 86 mg, $n = 16$). The ventricle was cut in half in the median plane. Two pieces of 5-0 silk were tied to each ventricle half, one to the remaining piece of *bulbus arteriosus* and the other to the apex. The preparation was placed in a water jacketed tissue bath at 15 °C containing Ringer's solution and bubbled with the above gas mixture. One piece of the

¹NaCl 125 mmol.L⁻¹, KCl 5.14 mmol.L⁻¹, NaH₂PO₄ 1 mmol.L⁻¹, MgSO₄ 0.94 mmol.L⁻¹, CaCl₂ 1 mmol.L⁻¹, NaHCO₃ 30 mmol.L⁻¹ and glucose 5 mmol.L⁻¹ Gesser *et al.* (1982).



Figures 3.1a&b. Diagram of the ventricle strip experiment. Ventricle strips (a1) were tied to a rubber bung (a2) and placed within a temperature controlled tissue bath (a3) with Ringer's solution. The opposite end of the ventricle strip was tied to the arm of a force transducer (a4) and the tissue given some tension by raising the transducer arm. The Ringer's was bubbled with a physiological gas mix (a5, see text for details). The tissue was stimulated to contract (five ms pulses of three - five mV, 12 pulses per minute) using an electrode (a6). Four trials were set up at a time (b1, one for each anaesthetic and one for a control). Signals from the force transducers were amplified (b2) and recorded on a chart recorder (b3).

silk was tied to the base of the tissue bath and the other to the arm of an isometric force transducer (1 g, Ugo Basile, model 7003). The transducer arm was raised until the silk was moderately taut but the ventricle not stretched, as it was found in preliminary experiments that stretching the ventricle caused the force of contractions to fall rapidly with time. The transducer was attached to a Gould transducer pre-amplifier and the signal sent to a chart recorder (Yokogawa, LR4110). Electrodes were placed either side of the ventricle and three to five mV pulses (five ms duration, 12 pulses/minute) were applied to stimulate the muscle to contract.

After a 15 minute equilibration period, anaesthetic was placed in the external Ringer's solution as a small, concentrated bolus which was mixed with the action of the bubbling gas. The anaesthetics tested, and their final bath concentrations, were MS222 (ten, 25, 50 and 100 ppm; unbuffered but the Ringer's solution contained a large amount of bicarbonate), metomidate (one, 2.5, five and ten ppm), and Aqui-S (six, 15, 30 and 60 ppm). These concentrations were 10%, 25%, 50% and 100% of the anaesthetic concentration needed to induce complete anaesthesia within about five minutes, in the fish species used. Each dose was left for 15 minutes before another concentrated anaesthetic bolus was added to increase the concentration to the next higher dose. Ventricle contractile force was recorded after 15 minutes exposure. After exposure to the last anaesthetic concentration the ventricle was washed by removing the anaesthetic contaminated Ringer's solution and replacing it with fresh solution. This was done at five minute intervals a total of three times, and the force of contraction measured after a total of 15 minutes. Controls were run in

parallel, where ventricle contractile force was measured every 15 minutes without the addition of anaesthetics.

Data were calculated as a percentage decrease from the maximum contractile force, this maximum arbitrarily being assigned 100%. A two-way ANOVA was used to compare the anaesthetics' effects at different concentrations. Tukey's HSD post-hoc test was used to further analyse data with primary interactions. $P \leq 0.05$ was used to indicate a significant difference. In all cases $n = 8$.

SERIES 3.2. Effects of MS222, metomidate and Aqui-S on the cardiac branch of the vagus and their ability to inotropically and chronotropically influence the heart *in situ*.

A diagram of the experimental set up for Series 3.1 is shown in Figure 3.2. Chinook salmon (mass 95 ± 12 g (2 SEMs), range 44.2 - 196.3 g, $n = 16$) were killed with a blow to the head and then pithed. A mid ventral incision was made from the cloaca to the isthmus and the heart exposed. The left operculum was removed, the gills on the left side sectioned, and the animal placed on its right side. The posterior cardinal vein was cannulated and perfusate was introduced immediately with a pressure of up to two cmH_2O . If perfusion of the heart did not start immediately the *sinus venosus* was cannulated via a hepatic vein. Inflow perfusate pressure was adjusted until the heart was pumping spontaneously and the *sinus venosus* was not over expanded. The ventral aorta was then cannulated and output pressure arbitrarily set at 47 cmH_2O . Input and output pressures were recorded with pressure transducers (Model 4-327, Bell and Howell Instruments Division, Pasadena, California) and the data amplified and recorded on a Devices MX4 recorder.

A one to two cm incision was made in the skin, along the line where the edge of the operculum meets the body, from a mid-lateral position to the top of the operculum. When pulled free from the muscle this skin formed a pocket at the base of which a branch of the vagus could be found. The pocket was immediately filled with Ringers solution (see above) to prevent desiccation and death of the nerve. The volume of the pocket was estimated at 200 - 400 μL . A platinum hook electrode, attached to a variable voltage and pulse rate stimulator, was placed under the nerve.

A range of voltages at 25 Hz was used to stimulate the vagus. Changes in heart rate or pressures could be observed from the output pressure chart. A voltage range (two volt maximum) was chosen from a voltage having no effect to a voltage producing a large effect. Anaesthetic was then placed in the pocket of skin. Over the next five minutes the same range of voltages was applied to the vagus and the effect on the heart rate and perfusate pressures observed. The anaesthetic in the pocket was then washed out thoroughly using three flushes of one mL of Ringer's solution. The

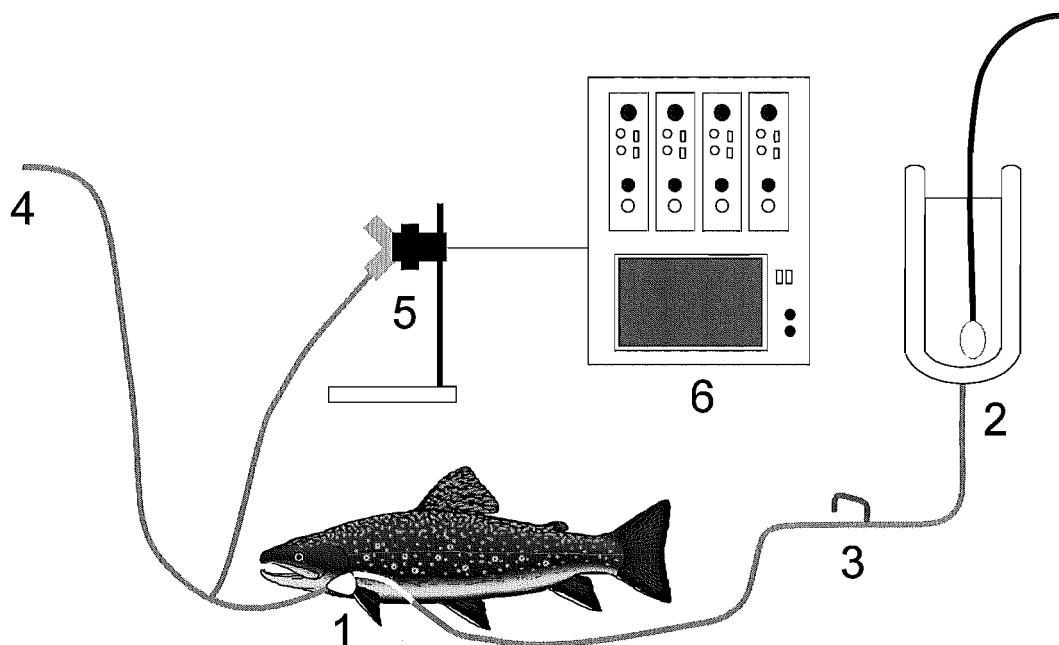


Figure 3.2. Diagram of *in situ* perfused heart preparation. Fish were killed and the heart exposed (1). The posterior cardinal vein or *sinus venosus* was cannulated and perfusion initiated from a water jacketed Ringer container. The Ringer's was bubbled with a physiological gas mixture (see text for details). Inflow pressure was regulated using a side branch from the cannula (3). The ventral aorta was then cannulated and the end of the cannula placed 47 cm above the heart (4). Branching off this cannula was a second cannula leading to a pressure transducer which monitored ventral aortic perfusate pressure (5). Signals from the pressure transducer were amplified and recorded using a Devices MX4 recorder (6).

same range of stimulation voltages was repeated. The anaesthetics tried were 100 ppm MS222, seven ppm metomidate and 60 ppm Aquí-S. These concentrations represent levels at which the Chinook salmon are anaesthetised within about five minutes. If no response occurred the concentration was increased by ten times and the stimulations tried again. This was repeated up to 100 times the original anaesthetic concentration.

The data were qualitative in that there was either an effect, no effect, or possibly an effect. An effect was defined as the requirement for an increase in voltage needed to elicit a particular change in heart rate or pressure that was also followed by a decrease in voltage required after washout. As the voltage required for particular effects changed spontaneously with time this was not always easy to determine.

RESULTS

SERIES 3.1. The effects of MS222, metomidate and Aqui-S on paced ventricle strips.

In every trial the ventricle strips continued beating for the duration of the experiment. However, there was some intrinsic decrease in contractile force with time. The control ventricle contractions were significantly lower at 45, 60, 75 and 90 minutes than at 15 minutes (Figure 3.3a).

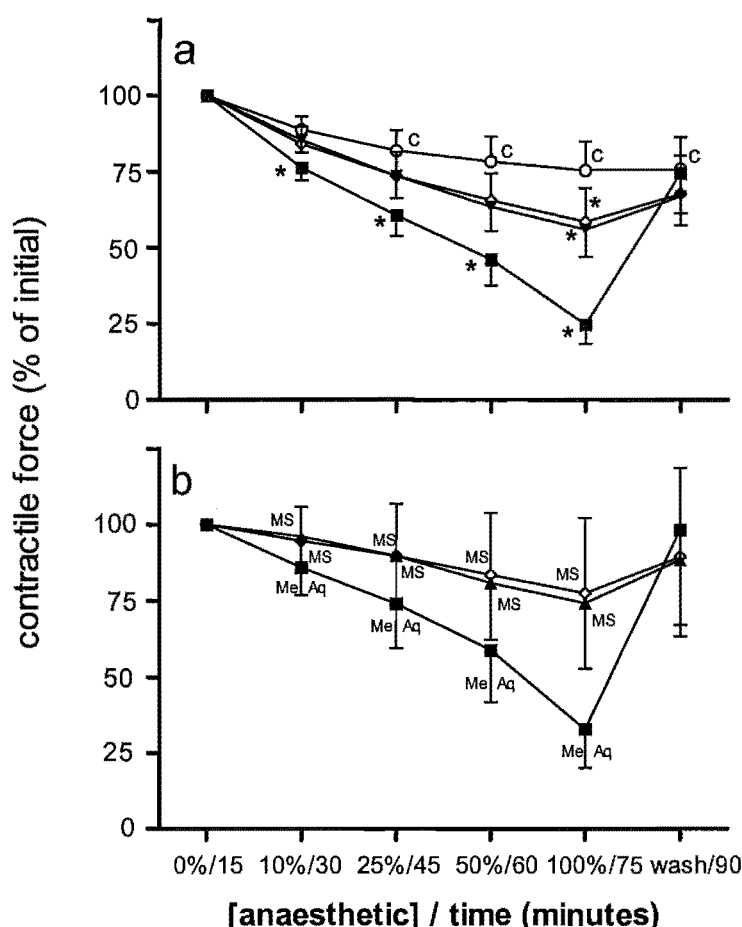
The change in ventricle contractility with increasing anaesthetic concentration and time can be seen in Figure 3.3a. The anaesthetic modified ventricle contractions were proportionally adjusted for the decrease in contractile force of the control strips (control strip values assigned as 100% contractile force) and these data were plotted in Figure 3.3b.

The two way ANOVA showed there was a significant interaction between anaesthetic and concentration. MS222 treated strips were significantly different from control strips at all concentrations while metomidate and Aqui-S treated strips were significantly different from controls only at 100% anaesthetic concentration. Although not all increases in anaesthetic concentration caused a statistically significant decrease in contractile force the data suggest a dose dependent effect of all of the anaesthetics on ventricle contractile force (Fig. 3.3a and b). MS222 treated ventricles had significantly lower contractile forces compared to metomidate and Aqui-S treated ventricles at all anaesthetic concentrations (Fig. 3.3b). This shows that MS222 has a much greater effect on the ventricle myocardium than either Aqui-S or metomidate. The contractile force of metomidate and Aqui-S treated ventricles was about $\frac{3}{4}$ of control values at full anaesthetic concentration, whereas MS222 treated strips had about $\frac{1}{3}$ the contractile force of the control strips.

After washing out the anaesthetics the contractile forces of all treated ventricle strips returned to control levels (Fig. 3.3a) which shows that the effects of all of the anaesthetics were reversible within 15 minutes if the anaesthetics were removed from the external medium.

SERIES 3.2. The effects of MS222, metomidate and Aqui-S on the cardiac branch of the vagus and its ability to inotropically and chronotropically influence the spontaneously beating heart.

If the cannulation and perfusion of the *in situ* hearts was performed quickly the preparations would last for at least one hour which was the time required to test all (up to 7) of the treatments. Delays in perfusion due to technical difficulty probably limited the useful life of a preparation. The mean number of treatments tested per heart was 4 ± 1 (2 SEMs, $n = 16$). The initial heart rate of the



Figures 3.3a&b. Graphs of proportional decrease in the contractile force of the ventricle with increased anaesthetic concentration or with time. The anaesthetics were MS222 (■), metomidate (▲) and Aquis-S (◇) and a control (○). (a) shows raw data and (b) shows data adjusted for changes in the control. Error bars are ± 2 SEMs. ^c = significant difference from control at 15 minutes. * = significant difference from the control value at the equivalent time. ^{MS}, ^{Me}, ^{Aq} = significant difference from MS222, metomidate, Aquis-S treated ventricles at the equivalent time.

preparations was 89 ± 6 beats per minute (2 SEMs, $n = 16$) whereas that of the whole fish were 50.8 ± 3.1 beats per minute (2 SEMs, $n = 63$, see Chapter 2, Results, Section 2.2). This difference is most likely due to temperature as the whole fish were maintained at 14°C whereas the perfused hearts were left moistened in air at temperatures of up to 20°C . The post-*bulbus arteriosus* pressure pulses were regular. With time, heart rate tended to decrease and pressure changes became more variable. Upon stimulation of the vagus a variety of different changes in heart rate and in post-*bulbus arteriosus* pressure were observed. For each fish the severity of the changes was voltage dependent although the exact nature of the changes and the voltage required to cause changes varied between preparations. Typical changes are shown in Figure 3.4. Figures 3.4 a&b show typical responses to a range of voltages. Figure 3.4c shows a maximal response where the heart did not beat until the stimulation was stopped. Figures 3.4d&e show some unusual changes.

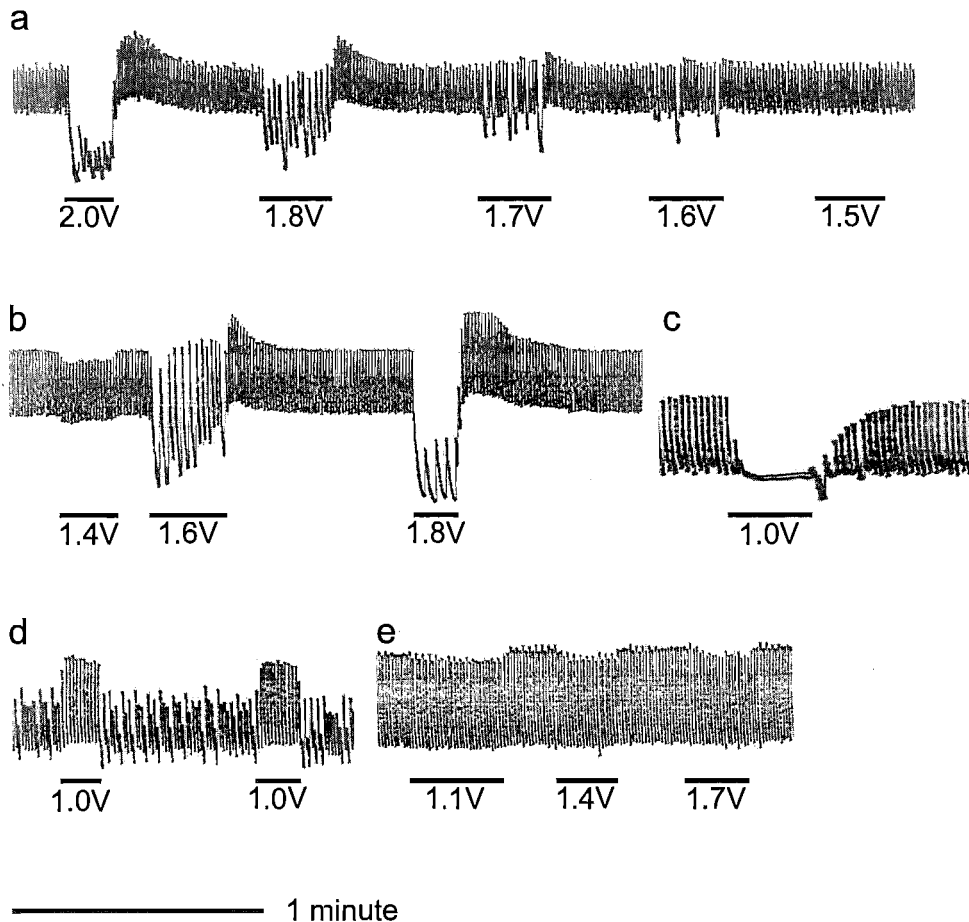


Figure 3.4a-e. Effect of electrical stimulation of a branch of the vagus nerve on output perfusate pressure in *in situ* spontaneously beating Chinook salmon hearts. (a) and (b) show typical responses to vagal stimulation with a range of voltages. Lower voltages generally caused a slight decrease in mean pressure and a slight decrease or irregularity in the heart rate. Higher voltages caused similar effects but of a greater magnitude. The greatest effect was a complete cessation of the heart beating (c). Less common responses included increasing the regularity and force of contractions (d) and a mild decrease in systolic pressure (e).

The effect of the three anaesthetics on the ability of the stimulated vagus to affect the spontaneously beating heart can be seen in Figure 3.5. The application of 100 ppm MS222 to the vagus increased the potential required to affect the heart. This effect was readily and quickly reversible after anaesthetic washout. A limited number of tests with 50 ppm MS222 had the same results. These data also suggest metomidate and Aqui-S could affect conduction down the vagus at 100 times their effective anaesthetic concentrations, but when an effect was seen recovery after washout was prolonged, required a greater number of washes and was incomplete. The results showed that MS222 affected neural transmission through the vagus at concentrations equal to and lower than its anaesthetic concentration whereas metomidate and Aqui-S do not.

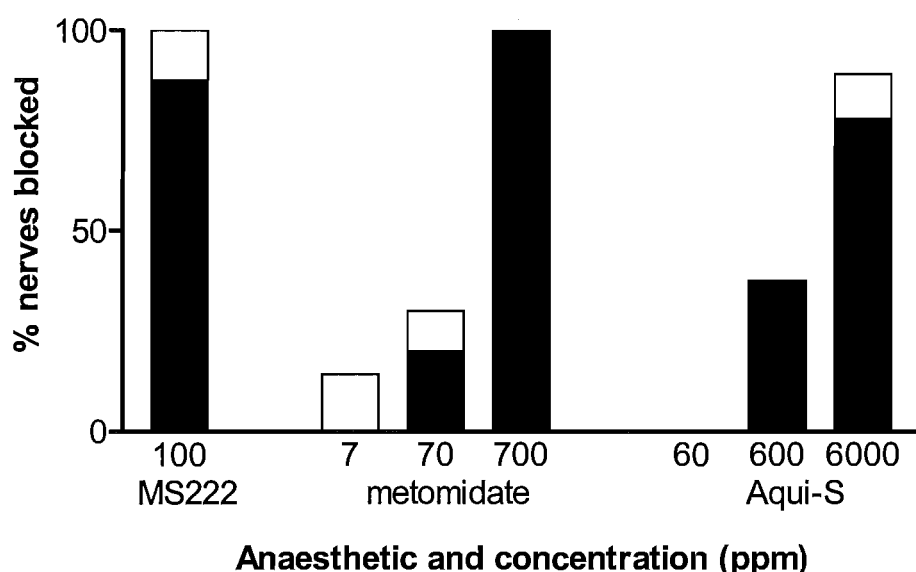


Figure 3.5. Graph of percent nerves affected by anaesthetic exposure versus anaesthetic and concentration. Black bar is definite effect and white bar is possible effect.

DISCUSSION

MS222, metomidate and Aqu-i-S all decreased myocardial contractility to some extent with MS222 having a greater effect than metomidate and Aqu-i-S. The effect of MS222 on Chinook salmon ventricle strips was very similar to its effect on the ventricle strips from rainbow trout (Ryan *et al.*, 1993). At MS222 bath concentrations of 25 ppm, 50 ppm and 100 ppm the decrease in contractility in this study was 40%, 54%, and 75% whereas in the study of Ryan *et al.* (1993) it was approximately 32%, 59% and 84% at concentrations of 27 ppm, 54 ppm and 108 ppm (data read from Fig. 1 in Ryan *et al.*, (1993)). This similarity is not unexpected considering the close phylogenetic relationship of the two fish species (both genus *Oncorhynchus*). The whole heart preparations of Ryan *et al.* (1993) showed quantitatively similar effects at comparable tissue concentrations of MS222. Similar results were observed in tench (*Tinca tinca*) isolated perfused hearts, where a decrease in the beat amplitude occurred after exposure to 30 - 60 ppm MS222 (Shelton and Randall, 1962).

The negative inotropic effects of metomidate and Aqu-i-S were quantitatively similar, and smaller than the effect of MS222. Analogues of the active component of Aqu-i-S (propanidid, propinal, estil), and metomidate and its analogues (etomidate, propoxate) are short or ultra-short acting intravenous anaesthetics or hypnotics in mammals (Hall and Clarke, 1991). Propinal was abandoned because of it causing, amongst other negative effects, severe circulatory problems

(Dundee and Clarke, 1979; Hall and Clarke, 1991). Propanidid, an anaesthetic not favoured due to cardiovascular effects (Hall and Clarke, 1991), has been shown to have negative inotropic effects on the hearts of dogs and guinea pigs (Reneman and Janssen, 1977). In contrast, etomidate had no effect on myocardium from these species or on isolated cat papillary muscle (Reneman and Janssen, 1977) and in textbooks is often noted for its minimal effects on the cardiovascular system (Aitkenhead and Smith, 1996; Dundee and Clarke, 1979; Hall and Clarke, 1991; Stoelting and Miller, 1989). However, etomidate has been shown to cause a 40.6% decrease in stroke volume and a 20.9% decrease in contractility in the heart of the cat using an isolated heart lung preparation (Fischer and Marquort, 1977). In humans etomidate causes a 10% increase in heart rate but has no other notable cardiovascular effects (Gooding and Corssen, 1976; Gooding and Corssen, 1977). There is less information on metomidate but one text stated that during metomidate anaesthesia the cardiovascular system is remarkably stable (Hall and Clarke, 1991). This information strongly suggests Aqui-S could have been expected to have had a greater effect than metomidate on the salmon myocardium, but this was not the case.

The greater effect of MS222 versus metomidate and Aqui-S is not unexpected if studies using mammals are considered. In mammals MS222 is a local anaesthetic and local anaesthetics affect all types of muscle. The negative inotropic effects of local anaesthetics in mammals are well documented as are the serious consequences of injecting significant amounts of local anaesthetic into the blood stream and affecting the heart and vascular smooth muscle (Hall and Clarke, 1991; McDonald and Wann, 1978). Local anaesthetics, as their name implies, act where they are applied whereas intravenous anaesthetics and hypnotics generally cause their primary effects by affecting the central nervous system. Therefore, *in vivo* negative inotropic effects of intravenous anaesthetics may be expected to be induced primarily via the central nervous system. Intravenous drugs, such as metomidate and analogues of the active component of Aqui-S, must have minimal deleterious effects on the cardiovascular system as they are often used on individuals that are unwell. So the lesser effects of metomidate and Aqui-S, compared to MS222, on the ventricle strips were probably to be expected. However, most mammalian general anaesthetics (Black, 1980; Hall and Clarke, 1991) and short acting intravenous narcotics such as propanidid, methohexital, ketamine and althesin (Fischer and Marquort, 1977) possess some undesirable cardiovascular side effects.

After washing out the anaesthetics the contractile forces of all treated ventricle strips returned to control levels (Fig 3.3a). This shows that the muscle relaxing effect of all three anaesthetics is reversible within 15 minutes if the anaesthetics are removed from the external medium. This may occur more slowly in the whole fish as removal of anaesthetic from the site of action may be prolonged. This may be exacerbated by a compromised heart as the heart is indirectly responsible

for removing anaesthetic from the site of action and for transporting the anaesthetic to the gills for excretion out of the body.

Although the ventricle strip experiment showed effects *in vitro*, *in vivo* effects cannot be implied from these data. The negative inotropic effects observed in the salmon ventricle may be countered with neural, hormonal and/or direct myogenic homeostatic responses (Laurent *et al.*, 1983) so that the net effect on the whole animals may be negligible (Fischer and Marquort, 1977). This would depend on the ability of the animal's homeostatic control to alter the response and also on the ability of the anaesthetising agent to block or attenuate any homeostatic response. Many anaesthetics are known to affect normal physiological responses. For example, local anaesthetics block nerves and metomidate and its analogues block a step in the production of cortisol (Olsen *et al.*, 1995). Also, halothane is thought to block the carotid baroreceptor mediated increase in heart rate in response to hypotension in humans (Stoelting and Miller, 1989). Apart from affecting the heart, fish anaesthetics may cause other side effects which could affect the cardiovascular system. In particular, many drugs that affect the heart are vasoactive, so resistance and hence blood pressure may be affected via other routes.

What effects would a reduced myocardial contractility have without homeostatic hormonal, neural or other anaesthetic modification? The fact that blood pressures, flow and vessel resistances alter simultaneously due to purely physical influences such as vessel distensibility and changes in blood viscosity (Wood, 1974) makes any suggestions speculative even without the added complexity of hormonal or neural homeostatic changes. A decreased contractile force implies that the maximum force a heart could produce would be reduced. This would be evidenced by a decrease in power output which has been demonstrated in the isolated rainbow trout heart exposed to MS222 (Ryan *et al.*, 1993). Isolated perfused hearts fail to deliver perfusate if the outflow pressure is too high (Forster, 1989, pers. obs). This occurs when the pressure produced within the contracting heart falls below the outflow pressure. Initially, this would be seen as a reduced period of time between opening and closing of the semilunar valves, between the ventricle and the *bulbus arteriosus*, which would result in a smaller ejection fraction, reduced stroke volume and a reduced cardiac output (Forster, 1989). If the afterload is sufficiently high in the *in situ* perfused heart the semilunar valves would not open. With a reduced heart contractile force, caused by an anaesthetic for example, the pressure able to be produced within the heart is reduced. *In vivo*, the pressure downstream would decrease rather than remain static, as pressure is a product of blood flow and resistance, and blood flow would decrease. This suggests blood pressure could also be affected by compromised myocardial contraction. The negative inotropic effects of local and some general anaesthetics (halothane, enflurane) have been implicated in a reduced aortic blood pressure in mammals (Black, 1980; Stoelting and Miller, 1989). The cause of death due to local anaesthetic

systemic toxicity is cardiovascular collapse caused by myocardial depression in conjunction with vasodilation (Aitkenhead and Smith, 1996). An impaired heart may cause fish additional problems during recovery. Anaesthesia is often a stressful procedure for fish whether it is caused by the actions of the anaesthetic or the actions of the anaesthetist. Stress in fish is generally accompanied by frantic activity, and both stress and activity necessitate an increased cardiac output produced by an increased stroke volume and/or heart rate. The reduced maximum cardiac output of an inotropically impaired heart in an aerobically challenged fish would decrease the time to exhaustion and prolong recovery from hypoxia and anaesthesia. As one of the main goals of anaesthesia is to mitigate stress, a stress free induction and an anaesthetic that did not cause a stress reaction would be advantageous.

Cardiac performance can be reduced not only by decreasing the force of contraction, as discussed above, but also by interfering with the pacing of contractions. MS222 increased the potential required to initiate and/or maintain a signal down the vagus nerve, a nerve which can decrease, and in some species of fish, increase heart rate (Farrell, 1992; Saito and Tenma, 1976; Satchell, 1991; Taylor, 1992). MS222 has been shown to affect other neural paths in fish. MS222 stopped the afferent activity of lateral line nerve fibres in cod (*Gadus gadus*) and the Ampullae of Lorenzini of dogfish (*Scyliorhinus canicula*) (Hensel *et al.*, 1975). Similarly, afferent activity from the lateral line to the medulla in *Tilapia leucosticta* and *Rutilus rutilus* decreased and the trigeminal nerve activity caused by physical stimulation of the skin decreased (Späth and Schweickert, 1977).

Local anaesthetics, like MS222, affect neurons by interfering with sodium, and possibly potassium, movement across the membranes of axons and reduce or abolish the ability of an electrical impulse to initiate an action potential and reduce the conduction velocity of action potentials (Brander *et al.*, 1982). There is some disagreement over whether local anaesthetics increase (Levy, 1974) or do not affect (Corvino, 1980; Stoelting and Miller, 1989) the excitation threshold potential. Work on squid axons showed that MS222 reversibly increases the excitation threshold and blocks nerve conduction (Frazier and Narahashi, 1975). In contrast to MS222, metomidate and Aqui-S could only block the vagus nerve at extreme concentrations unlikely to be reproduced *in vivo*. Action potentials can be blocked by inert gases, general anaesthetics, barbiturates and alcohols (McDonald and Wann, 1978) although the concentrations required to block action potentials may be greater than the effective anaesthetic concentration. Etomidate has been shown to affect ion movement in frog (*Rana esculenta*) myelinated nerve fibres but the minimum concentration used was 24 ppm (Benoit, 1995). This concentration is at least six times higher than the concentration required to cause anaesthesia in fish (Summerfelt and Smith, 1990). Etomidate has no effect on the nerves of dog and guinea pig hearts at the anaesthetic concentrations generally used (Reneman and Janssen, 1977). The mechanism of action of these chemicals is

unknown but it is generally thought that general anaesthetics affect membrane proteins of cells within the central nervous system to cause anaesthesia or hypnosis (Richards, 1980). They may affect neurons similarly, by attaching to receptor sites on the channels proteins (Benoit, 1995).

Although MS222 can affect the vagus at 100 ppm, a typical anaesthetic bath concentration, for it to have an effect *in vivo* this concentration must occur at the site of the nerve. Accumulation studies of MS222 in fish suggests this does occur. Hunn (1970) found that eight species of fish all accumulated more than 100 ppm MS222 in their brain after five minutes exposure to MS222. Similar high concentrations of MS222 were found in the brain of *Pagrus auratus* (Ryan, 1992b). This shows fish exposed to 100 ppm MS222 do accumulate enough MS222 in the brain to have an effect on the vagus. No accumulation studies have been performed on fish using metomidate, or Aqui-S, or any of their analogues, so the rate of accumulation and the maximum attainable concentrations for these chemicals are unknown. However, the present study suggests very high concentrations of both metomidate and Aqui-S are required in order for peripheral nerve inhibition to occur, and accumulation of such a concentration of anaesthetic is unlikely.

A number of studies suggest MS222 can act both centrally and peripherally. It has been suggested that there is a rapid depression of central autonomic control centres including tonic cardiovagal inhibition (Houston *et al.*, 1971a). Evidence for possible central effects of MS222 comes from previous nerve studies. It has been shown that the phasic response of sound sensitive neurons in the medulla of *Tilapia leucosticta* and *Rutilus rutilus* to sound stimuli ceased during MS222 anaesthesia and also that the afferent output from the medulla to the lateral line was decreased by 70% (Späth and Schweickert, 1977). These studies support the idea that MS222 has central effects. Metomidate and Aqui-S are intravenous anaesthetics and as such their primary site of action is assumed to be the central nervous system.

The ability of MS222 to block the vagus nerve *in vitro* does not elucidate what will happen in the whole fish. As suggested above, direct myogenic, hormonal or other neural homeostatic responses could neutralise the effects observed *in vitro*. In *Oncorhynchus mykiss* a reduced heart rate after 30 minutes of 100 ppm MS222 anaesthesia was attributed to maximum cholinergic vagal inhibition, the supporting evidence being an increase in heart rate after intraperitoneal and/or intrapericardial injections of atropine (Lochowitz *et al.*, 1974). This suggests MS222 was not blocking the vagus or inhibiting production of the negative chronotropic tonus centrally, even though MS222 concentrations should be high enough in the brain (Hunn, 1970; Ryan, 1992b) and vagus. MS222 in the ventilation stream of tench (*Tinca tinca*), with bilaterally sectioned tenth vagi, caused the heart rate to decrease (Randall, 1962) which suggests that a decreasing heart rate during MS222 anaesthesia is not necessarily caused by autonomic depression. However, in dogfish

(*Scylliorhynchus canicula*) sectioning of cranial nerves IX and X were required to abolish a hypoxic bradycardia (Butler *et al.*, 1977) which suggests the response seen in the experiment of Randall (1962) could have been a neural inhibitory effect.

In contrast to the lack of effect of MS222 on the negative chronotropic aspect of the vagus, MS222 and benzocaine may affect the branches of the vagus that have a positive chronotropic effect on the heart. Attempts to increase the rate of the heart of parore (*Girella tricuspidata*) anaesthetised with MS222 and benzocaine, by stimulating the sympathetic innervation in the atropinized vagus, failed, whereas the hearts of fish not subjected to MS222 or benzocaine anaesthesia did show an increased heart rate upon stimulation of the atropinized vagus (Montgomery *et al.*, 1986). This suggests MS222 may have a selective effect upon the vagus with the sympathetic nerves being more susceptible than the parasympathetic nerves. The sensitivity of neurons to local anaesthetics tend to follow the general rules that myelinated nerves are more sensitive than nonmyelinated nerves, smaller diameter nerves are more sensitive than larger diameter nerves and active nerves are more sensitive than inactive nerves (Rowland, 1974; Stoelting and Miller, 1989). Vagal preganglionic cranial autonomic neurons are thought to consist of small myelinated fibres (Laurent *et al.*, 1983) which would be prime candidates for blocking with MS222 using the criteria above. The sympathetic nerves are thought to be a part of a group of nonmyelinated fibres of various sizes and, as they are non-myelinated, their sensitivity to MS222 may be lower. Unfortunately, these neuron dependent sensitivities contradict what is occurring in whole fish as they suggest the negative chronotropic effect should be blocked before the positive chronotropic effect.

Unlike an axon, the vagus nerve does not show an “all or nothing” response because it is a nerve bundle. There was evidence for this in Series 3.2 as application of higher potentials to an MS222 affected vagus could increase the response of the heart. Theoretically, an exogenous agent could have a graded inhibitory response depending on how many nerve fibres were affected. The magnitude of the effect on a nerve would be determined by the sensitivity of different neurons within the bundle (mentioned above) and also the penetration of the agent into the bundle. The amount of penetration of a local anaesthetic is affected by the agent (dissociation constant, lipophilicity), the solution (pH, ionic concentration), and the nerve bundle morphology (anatomy, perfusion, adjacent tissue) (Stoelting and Miller, 1989). The inability of Aqui-S and metomidate to affect the vagus nerve could be controlled by these factors. Similarly, these factors could modify how these anaesthetics affect groups of muscle fibres.

Apart from its ability to block conduction down axons MS222 has been shown to reduce transmitter release at the neuromuscular junction using frog (*Rana pipiens*) sartorius muscle preparations (Maeno, 1966). Maeno (1966) found that 2.6 ppm (10^{-5} M) MS222 significantly reduced the mean

quantal content of end plate potentials, but did not change the stimulation threshold and had only a small effect on the electrical activity of the muscle fibre. Higher concentrations eventually abolished excitability of the muscle fibre membranes. In the present study the anaesthetic solutions were placed in a pocket, made by pulling some skin away from some skeletal muscle, at the base of which occurred a branch of the vagus. It is possible, although unlikely, that anaesthetic solution washed out from this pocket may have fallen onto the heart and affected the nerve muscle interface. Maeno (1966) also noted that penetration of MS222 into muscle tissue was slow as even after ten to 20 minutes 26 ppm (10^{-4} M) MS222 had not penetrated to muscle fibres three to four layers below the surface. It is possible that in the whole animal the neuromuscular junctions are protected by surrounding tissue although a good blood supply to the area could lessen any protective effect of surrounding tissue.

Carp (*Cyprinus carpio*) under prolonged MS222 anaesthesia eventually showed atrioventricular dissociation which the authors attributed to hypoxia (Serfaty *et al.*, 1959). In the present study this was noted in *in situ* perfused hearts as they were beginning to fail and die. It has been noted that some local anaesthetics can also cause atrioventricular conduction blocks in human patients as well as arrhythmogenesis (McCaughey, 1992). Ryan *et al.* (1993) found that ten ppm MS222 caused arrhythmia and complete arrest of the whole heart of rainbow trout.

Electrocardiograms (ECGs) directly measure electrical activity in the heart and are therefore useful for monitoring changes in electrical activity that occur with anaesthesia. ECGs have been measured in fish undergoing anaesthesia. In general, few notable changes were observed in the ECGs of fish under going anaesthesia (see Campbell and Davies, 1963; McFarland, 1959). Where changes did occur the anaesthesia was prolonged (at least 30 minutes) and the changes were likely to be due to hypoxia (see Fredricks *et al.*, 1993; Yamamitsu and Itazawa, 1988). Both studies reported an increase in time between the QRS complex, which represents ventricular depolarisation, and the T wave, which represents ventricular repolarisation. Although these studies do not present solid evidence of anaesthetic effects on the electrical conduction in the hearts of fish, local anaesthetics have been implicated in widening of the QRS complex in human patients (McCaughey, 1992). This effect was also noted in carp (*Cyprinus carpio*) anaesthetised for two hours with 800 ppm 2-phenoxyethanol (Yamamitsu and Itazawa, 1988) but, again, separation of anaesthetic effects from the effects of hypoxia is difficult. In this study no electrocardiography was performed, and were it to be done in the future, with suitably designed experiments, ECGs might indicate direct effects on electrical conduction through fish hearts.

Chapter 4

Acute effects of MS222, metomidate and Aqui-S on the isolated perfused fish gills and isolated afferent and efferent branchial arteries of teleost fish

INTRODUCTION

The fish gill fulfils a number of vital functions including ion regulation and respiration. The gills receive all the blood immediately after it is ejected from the heart and must ensure that this blood is oxygenated adequately before it enters the systemic circulation. Fish gill haemodynamics are controlled by hormonal, neural, local and physical parameters (Wood, 1974) which can react to numerous exogenous and endogenous changes in order to maintain gill function. Changing gill haemodynamics with exogenous compounds is potentially detrimental to the fish. Anaesthetics, and local anaesthetics in particular, are known to affect vasoactivity in mammals (Hall and Clarke, 1991). Vasoconstriction or vasodilation can alter the pattern of blood flow through gill filaments and lamellae (Farrell *et al.*, 1979). This may in turn influence respiratory gas and ion exchanges (O_2 , CO_2/HCO_3^-) and could lead to hypoxia and/or hypercapnia.

The experiments in this chapter examine the direct chemical effect of MS222, metomidate and Aqui-S on fish gills. In the first experiment an isolated, perfused holobranch of the spotty (*Notolabrus celidotus*) was used to test whether the three anaesthetics under investigation would alter branchial vascular resistance and the partitioning of efferent flow. Altered vascular resistance would manifest as a change in afferent blood pressure measured at a constant flow rate. The response to the anaesthetics was contrasted with the predictable response to known vasoactive agents 5-hydroxytryptamine and the α -adrenergic receptor blocker phentolamine (in the presence of adrenaline). In the second and third experiments myography was used to examine the direct effects of MS222, metomidate and Aqui-S on vasoactivity using afferent and efferent branchial arteries from the spotty (*Notolabrus celidotus*) and the Chinook salmon (*Oncorhynchus tshawytscha*).

MATERIALS AND METHODS

SERIES 4.1. Acute effects of MS222, metomidate and AQUI-S on isolated perfused gills of spotties (*Notolabrus celidotus*).

Spotties were caught in traps in Lyttleton Harbour, South Island, New Zealand and transported to the University of Canterbury Zoology department. They were placed in a recirculating seawater system (ten to 15 °C, 12 hr/12 hr artificial light/dark regime) and fed mussels (*Perna canaliculus*) two to three times per week.

When required for experimentation the spotties (mass 86 ± 12 g (2 SEMs), range 50.0 - 120.3 g, $n = 10$) were killed with a blow to the head and their blood heparinized via the caudal vein using a heparinized seawater fish Ringer's solution¹. After a few minutes the gills were dissected out and placed in ice cold Ringer's solution until required.

Gills were cannulated via the afferent branchial artery and perfused with Ringer's solution (as above but with 10^{-7} mol.L⁻¹ adrenaline, kept at ten °C and gassed with 8.3% O₂, 91.2% N₂ and 0.5% CO₂). Adrenaline was added to the Ringer's solution to promote gill perfusion by causing branchial vasodilation, and so phentolamine could be used to try to effect a vasodilation by blocking α -adrenergic vasoconstriction. The perfusate was pumped into the gills at a constant rate of 110 μ L.min⁻¹ (about 50 - 70 % of normal afferent flow for an 86 g fish assuming cardiac output is 15 - 20 mL.kg⁻¹.min⁻¹) using a Gilson Minipuls 3 peristaltic pump. The efferent branchial artery was then cannulated and the end of the cannula placed 25 cm above the gill (ie. post-branchial afterload = 25 cmH₂O). This is a little lower than the average teleost dorsal aortic pressure of about 41 cmH₂O (see Satchell, 1991). The prepared gills were then placed in a perspex chamber filled with Ringer's solution (see Figure 4.1 for a diagram of the experimental set up). The chamber had an aperture for introducing drugs into the Ringer's solution around the gill, and an outflow pipe to release perfusate entering the chamber from the arterio-venous blood route. Prebranchial pressures were measured with blood pressure transducers (Model 4-327, Bell and Howell Instruments Division, Pasadena, California) and the signal amplified with Gould transducer preamplifiers. The efferent arterial flow was calculated by multiplying drop rate (measured using isometric levers as drop counters) by drop volume (estimated from drop mass). All data, except drop weights, were recorded on a LR4110 Yokogawa chart recorder.

¹ NaCl 171.1 mmol.L⁻¹, KCl 4.0 mmol.L⁻¹, NaH₂PO₄.2H₂O 3.3 mmol.L⁻¹, MgSO₄.7H₂O 1.2 mmol.L⁻¹, CaCl₂.2H₂O 1.5 mmol.L⁻¹, NaHCO₃ 15.0 mmol.L⁻¹, glucose 5.6 mmol.L⁻¹; modified from Shuttleworth *et al.* (1974) cited in Farrell *et al.* (1979)

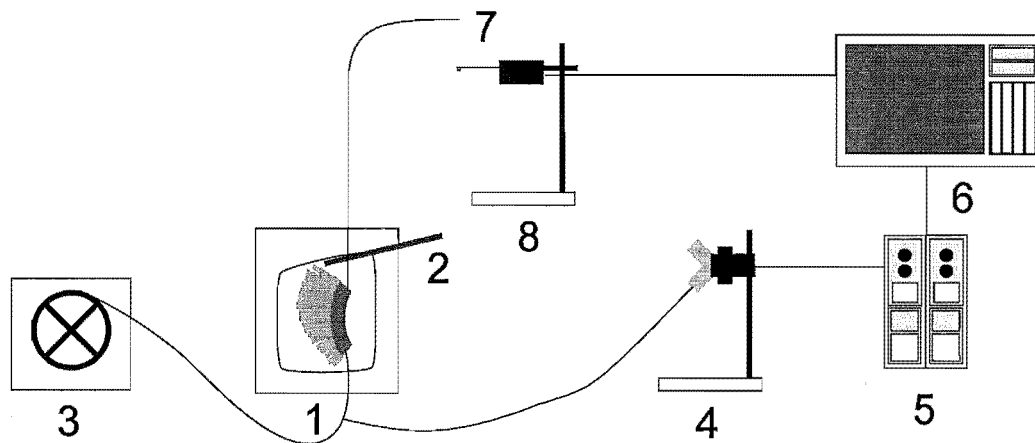
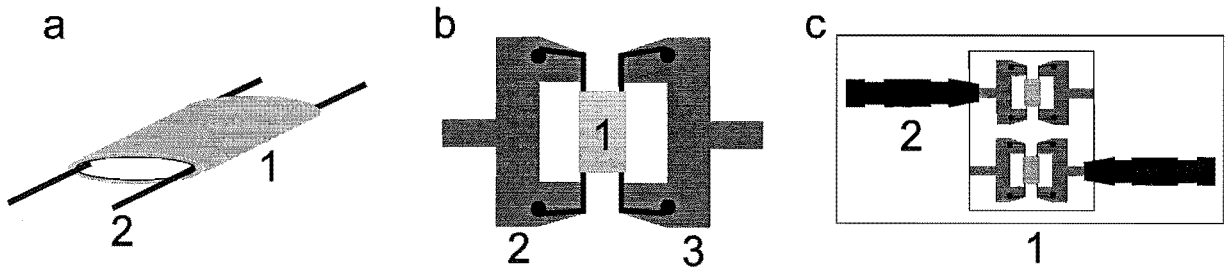


Figure 4.1. Diagram of the perfused gill set up. After cannulation of the afferent and efferent branchial arteries the isolated gill was sealed in a perspex box (1). The box had four holes, two for the cannulae, one for the introduction of anaesthetics (not shown) and one for the release of perfusate from the arteriovenous route (2). Perfusate was pumped (3) into the gill via the cannula in the afferent branchial artery. A second cannula branched off to a pressure transducer (4). The signal from the pressure transducer was amplified (5) and recorded on a chart recorder (6). The end of efferent cannula was placed 25 cm above the gill (7). A force transducer (8) acted as a drop counter and measured efferent flow. These data were also recorded by the chart recorder (6).

Anaesthetic was placed into the external Ringer's solution as a small, concentrated bolus. The anaesthetics tested, and their final external concentrations, were MS222 (ten, 100, 1000 ppm; unbuffered), metomidate (one, ten, 100 ppm), and Aqui-S (six, 60, 600 ppm). These concentrations were 10 %, 100 % and 1000 % of the concentration needed to induce complete anaesthesia in spotties within about five minutes. 5-hydroxytryptamine (Sigma Chemical Co.) and phentolamine were subsequently used to test the reactivity of the gills, and were introduced via the perfusate. 5-hydroxytryptamine causes vasoconstriction of sphincters in the efferent filamental arteries which causes an increase in branchial pressure. The concentration of 5-hydroxytryptamine applied was sufficient to stimulate any 5-hydroxytryptamine receptors present on the gill (Sundin *et al.*, 1995). Phentolamine, as mentioned above, is an α -adrenergic receptor blocker that should relax any α -adrenergic vasoconstriction. Phentolamine is a competitive blocker of adrenaline and so was used at a 100 times the concentration of adrenaline in the perfusate.



Figures 4.2a-c. Diagrams of myography set up. A two mm length of vessel (a1) was threaded with two lengths of thin wire (a2). This was placed between the arms of the myograph (b1) and then one wire attached to a movable arm (b2) and the other to a force transducer arm (b3) with two pairs of screws. Two vessels could be set up in the Ringer bath of each myograph (c1). Tension was placed on the vessels using vernier screws (c2) and the tension monitored on the myograph meter (not shown).

SERIES 4.2. The acute effects of MS222, metomidate and Aqualun S on the vasoactivity of teleost branchial arteries.

Experiment 4.2a. Myography of spotty efferent and afferent branchial arteries.

Spotties were obtained and held as above. When required, spotties (mass 105 ± 19 g (2SEMs), range 58 - 161 g, $n = 12$) were killed with a blow to the head and their blood heparinized via the caudal vein using a heparinized Ringer's solution (formula as in Series 4.1 above). After a few minutes the afferent or efferent branchial arteries were dissected out and placed in Ringer's solution until required (see Agassiz and Vogt, 1845, for diagrams of branchial vessels).

A diagram of the experimental set up is shown in Figure 4.2. Two mm lengths of vessel were mounted in myographs (myograph model 400A, myo-interface model 410A, J.P. Trading, Aarhus, Denmark) in Ringer's solution at 15°C . Tension was placed on the vessels and they were left for one hour to equilibrate. A gas mixture (0.5 % CO_2 in air) was bubbled through the Ringer's solution during this time. Four vessels were set up, three were exposed to one of each of the anaesthetics and the other was used as a control. Vessels were assigned treatments randomly.

After equilibration the anaesthetics were placed in the external Ringer's solution as a small, concentrated bolus and were mixed thoroughly with a Pasteur pipette. The anaesthetics tested were; MS222 (ten, 100, 1000 ppm; unbuffered), metomidate (one, ten, 100 ppm), and Aqualun S (six, 60, 600 ppm). The concentrations used were 10 %, 100 % and 1000 % of the concentration needed to induce complete anaesthesia in about five minutes. Each dose was left for 15 minutes before the next highest dose was added. Vessel tension was recorded after 15 minutes exposure.

Experiment 4.2b. Myography of salmon efferent and afferent branchial arteries.

Chinook salmon were purchased from a local farm (Isaac's Salmon) and transported to the University of Canterbury. They were placed in a large (1.5m³) tank with flow through artesian well water. The animals did not feed and were kept for up to one month.

The methods and materials for this experiment were identical to those for the spotty vessels except for the following changes. The salmon used had a mean mass of 359 ± 289 g (2 SEMs), range 78 - 1362 g, n = 17. The Ringer's solution² used was for freshwater fish. The range of concentrations of all of the anaesthetics was decreased to try and get a progressive response. The base concentration of metomidate was lowered (from ten ppm to seven ppm) as seven ppm was found to induce and maintain anaesthesia at a rate and level more similar to 100 ppm MS222 and 60 ppm Aqui-S. The anaesthetics used were; MS222 (ten, 25, 50, 100 ppm; unbuffered), metomidate (0.7, 1.75, 3.5, seven ppm), and Aqui-S (six, 15, 30, 60 ppm). The concentrations tested were 10 %, 25 %, 50 % and 100 % of the concentrations needed to induce complete anaesthesia in about five minutes.

For each of the Series 4.2 experiments a two-way repeated measures ANOVA and Tukey's HSD post-hoc test was used to compare the anaesthetics and doses of anaesthetic on the branchial vessels. Proportional changes were used as initial tension differed between anaesthetics, prior to any treatment. Responses of the two species were compared using the same tests but using only the 10 % and 100 % concentration data. The difference between the vasoactivity of the afferent and efferent vessels was also compared using the same tests but the control vessel data was excluded, as this measured only spontaneous changes and not anaesthetic induced changes. In all cases $P \leq 0.05$ was used to indicate a significant difference.

RESULTS

SERIES 4.1. Acute effects of MS222, metomidate and Aqui-S on isolated perfused gills of spotties (*Notolabrus celidotus*).

Perfused gill preparations are difficult to work with (Perry and Farrell, 1989). Setting up this preparation proved difficult and only nine of the 15 preparations maintained an efferent flow for the

² NaCl 125 mmol.L⁻¹, KCl 5.14 mmol.L⁻¹, NaH₂PO₄ 1 mmol.L⁻¹, MgSO₄ 0.94 mmol.L⁻¹, CaCl₂ 1 mmol.L⁻¹, NaHCO₃ 30 mmol.L⁻¹ and glucose 5 mmol.L⁻¹ from Gesser *et al.* (1982)

entire experimental period. The mean efferent flow was 23 ± 11 % of the afferent flow ($110 \mu\text{L}\cdot\text{min}^{-1}$). This is low and indicates that perfusate leaked from the junction between the cannula and the gill and from the cut ends of the holobranch. This is a common finding with such preparations and leads to an overestimate of the venous outflow (Perry and Farrell, 1989). The average initial prebranchial pressure for the gills was 99 ± 11 cmH₂O (2 SEMs, $n = 18$) which is about 2.5 times higher than that measured in teleosts *in vivo* (Satchell, 1991).

All but one of the 15 gills tested showed vasoconstriction in response to 5-hydroxytryptamine at a concentration of 10^{-7} mol.L⁻¹. The unreactive gill reacted to 10^{-6} mol.L⁻¹ 5-hydroxytryptamine. This indicates that the smooth muscle of the vessels in all the gills tested could contract and could respond to a vasoconstricting substance. No significant vasodilation was observed in response to adrenaline in the presence of phentolamine. This suggests the vessels may not have been able to dilate at the perfusion pressure used, as most teleost gills have vasodilator β -adrenergic receptors (Nilsson, 1984a). Neither drug caused any significant change in efferent flow, which would be expected in the case of the 5-hydroxytryptamine induced increase in pre-branchial pressure. This places some doubt on the accuracy of the latter result.

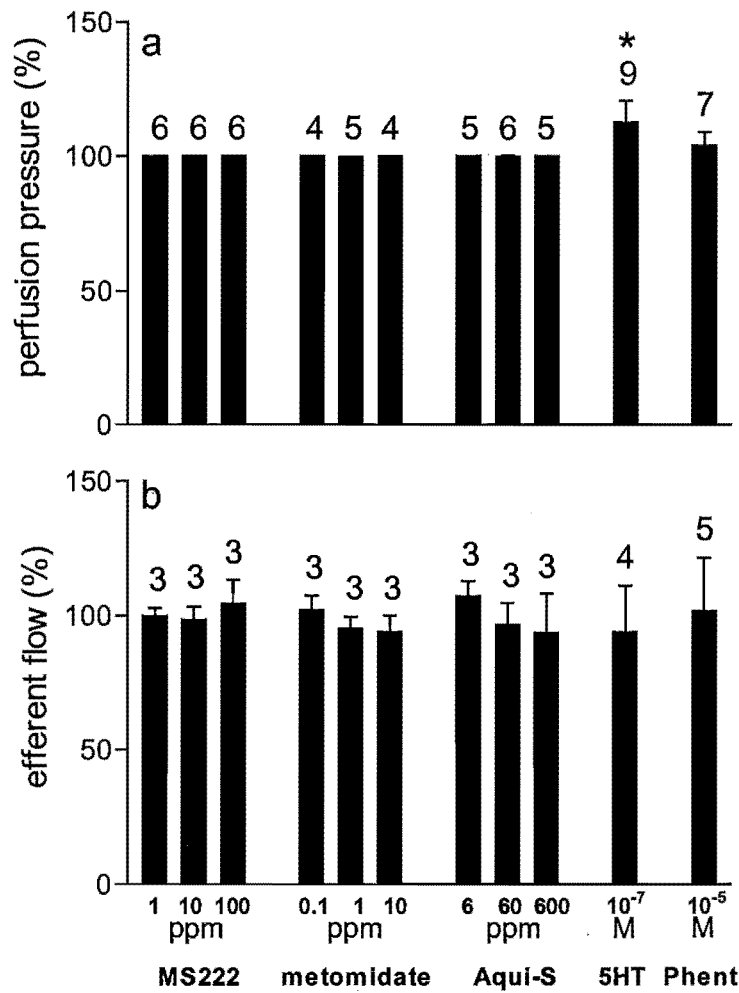
None of the gills showed any significant change in prebranchial pressure or efferent flow (Figures 4.3a&b respectively) in response to any of the anaesthetics at any of the concentrations tested. This suggests that none of the anaesthetics affected vasoactivity of the isolated perfused spotty gill. However, the ability of the preparation to detect changes must be in some doubt due to the ambiguous results shown by the control drugs (5-hydroxytryptamine and phentolamine).

SERIES 4.2. The acute effects of MS222, metomidate and Aqui-S on the vasoactivity of teleost branchial arteries.

Experiment 4.2a. Myography of spotty efferent and afferent branchial arteries.

Data showing the change in branchial artery tension with increasing concentration of anaesthetics for the spotty efferent and afferent vessels is plotted in Figures 4.4a&b.

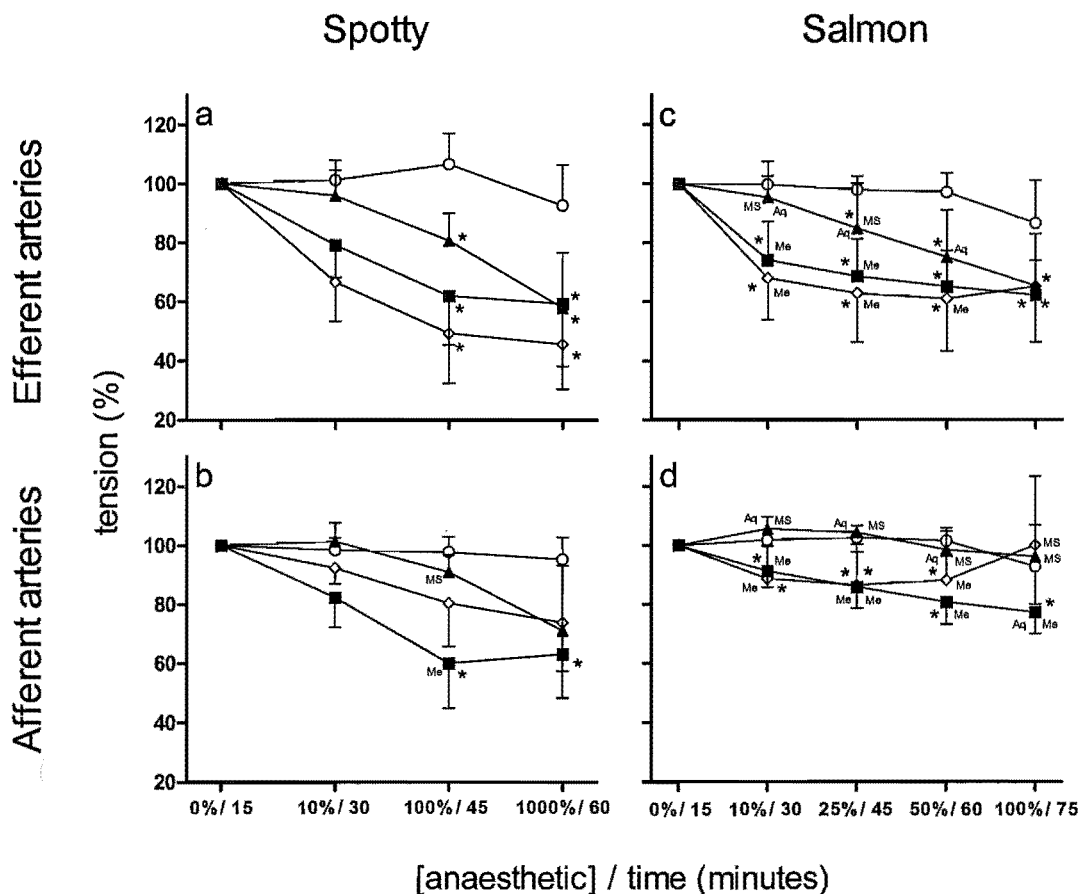
The mean starting tensions for the efferent and afferent branchial arteries, after the one hour equilibration period, were 1.52 ± 0.35 mN (2 SEMs, $n = 6$) and 1.87 ± 0.32 mN (2 SEMs, $n = 6$) respectively. The starting tensions of the efferent and afferent vessels were not significantly different. The tension of the control vessels did not change over the duration of the experiment,



Figures 4.3a&b. Percent change in prebranchial pressure (a) and percent change in efferent perfusate flow (b) from spotty gills in response to the drugs stated. The mean of the control values was arbitrarily assigned 100%. Error bars are ± 2 SEMs. The number above each bar is n. * indicates a significant difference ($P \leq 0.05$).

although the variation tended to increase with time, which shows the preparations were quite stable.

Analysis of the spotty efferent vessel data showed there was an interaction between anaesthetic type and concentration. The post-hoc test showed that all three anaesthetics at 100 % and 1000 % concentrations caused a significant reduction in vessel tension compared to the control. There were no significant differences between the anaesthetics (Fig. 4.4a). Analysis of the spotty afferent vessels showed there was an interaction between anaesthetic type and concentration. The post-hoc test showed MS222 was different from control vessels at 100 % and 1000 % concentrations and MS222 was different from metomidate at 100 % concentration. Overall, spotty efferent vessels had a greater decrease in tension than spotty afferent vessels in the presence of all anaesthetics ($p = 0.015$).



Figures 4.4a-d. Graphs of proportional change in tension of spotty efferent (graph a, $n = 6$) and afferent (graph b, $n = 6$) and salmon efferent (graph c, $n = 9$) and afferent (graph d, $n = 8$) branchial arteries in the presence of MS222 (■), metomidate (▲) and AQUI-S (◇) and the controls (○). Error bars are ± 2 SEMs. * = significant difference from the control value at the equivalent time. ^{MS}, ^{Me}, ^{Aq} = significant difference from MS222, metomidate, AQUI-S treated branchial vessels at the equivalent time. Note that 100 % metomidate is ten ppm for spotties and seven ppm for salmon.

Experiment 4.2b. Myography of salmon efferent and afferent branchial arteries

Data showing the change in branchial artery tension with increasing concentration of anaesthetics for the salmon efferent and afferent vessels can be seen plotted in Figures 4.4c&d.

The mean starting tensions for the efferent and afferent branchial arteries, after the one hour equilibration period, were 2.34 ± 0.49 mN (2 SEMs, $n = 9$) and 1.73 ± 0.32 mN (2 SEMs, $n = 8$) respectively. These starting tensions are significantly different. However, if an outlier (greater than 2 standard deviations from the mean) from a fish which was much heavier than the rest (mass of 1362 g vs. a mean of 359 ± 289 g (2 SEMs) for all the salmon) is removed the difference between the initial starting tensions of the efferent and afferent branchial arteries is not significant. As with the spotty vessels, the tension of the salmon control vessels did not change over the duration of

the experiment. Similarly the variation in tension tended to increase with time, which shows the preparation was again quite stable.

The effects of the anaesthetics on the salmon branchial vessels were similar to the effects seen on the spotty branchial vessels. The analysis of the efferent vessel data showed an interaction between anaesthetic type and concentration. The post-hoc test showed that all anaesthetic exposed efferent vessels, except those exposed to 10 % metomidate, had tensions lower than the equivalent control vessels (Fig. 4.4c). MS222 and Aqui-S treated efferent vessels behaved the same, whereas metomidate caused a smaller drop in tension at lower concentrations (metomidate treated vessel tensions were higher than Aqui-S treated vessel tensions at 10 %, 25 % and 50 %; and higher than MS222 treated vessel tensions at 10 % and 25 %). The salmon afferent vessel data also showed an interaction between anaesthetic type and concentration. All MS222 treated afferent vessels had lower tensions than control vessels, metomidate had no apparent effect, and 10 %, 25 % and 50 % Aqui-S treated vessels had lower tensions than the equivalent control vessels. Of note is the apparent increase in tension of Aqui-S treated vessels at higher concentrations. As in the spotty, the salmon efferent vessels were affected by the anaesthetics more than the afferent vessels.

Analysis of the 10 % and 100 % data of the two species show that the branchial vessels of both species behaved similarly in the presence of the anaesthetics.

To summarize, most of the anaesthetics had a vasodilator effect on both the efferent and afferent vessels of both species at one or more of the concentrations tested. The exceptions to this generalization are the increase in salmon afferent branchial artery tension in the presence of the highest dose of Aqui-S, and the lack of effect of metomidate on the same vessels. MS222 and Aqui-S tended to act similarly and cause greater relaxation of vessels than metomidate. Efferent vessels were affected more than afferent vessels and the vessels of spotties and salmon reacted similarly.

DISCUSSION

The perfused gill experiment suggests that none of the fish anaesthetics affect gill blood flow. A previous study found that 100 ppm MS222 decreased the efferent flow of isolated perfused rainbow trout gills by about $\frac{1}{3}$ (Fromm *et al.*, 1971). However, methodological problems in the present study and the study of Fromm *et al.* (1971) make the interpretation of results difficult. Fish gill perfusion is modulated neurally, hormonally, locally and physically, and balancing these effects is difficult

experimentally. An understanding of the vasculature and function of the fish gill is required to explain possible deficiencies in the perfused gill experiment in both this study and in the study of Fromm *et al.* (1971).

The fish gill circulation consists of three separate pathways (Satchell, 1991). The arterio-arterial path is the largest by volume and directs blood from the ventral aorta, through the gills where it is oxygenated, and then to the dorsal aorta for distribution around the body. The second and third pathways, the gill nutritive vessels and the interlamellar vessels, make up the arteriovenous system. Only 7 % of the afferent branchial blood flow is diverted to the central venous sinus of the rainbow trout (*Oncorhynchus mykiss*) (Ishiamitsu *et al.*, 1988). The gill nutritive system supplies cells deeply embedded within the gills with nutrients and oxygen (analogous to the coronary circulation of the heart) and the interlamellar vessels are thought to supply chloride cells (Satchell, 1991). The gill nutritive vessels branch off the arterio-arterial system from the efferent filamental arteries and the interlamellar vessels branch off the gill nutritive vessels.

Blood enters the gills from the ventral aorta via the afferent branchial arteries. This blood is at a high pressure having only passed through the *bulbus arteriosus* and ventral aorta after being ejected from the ventricle. Blood moves up the gill arch in the afferent branchial artery and then moves into the primary filaments via the afferent filamental arteries which run along the trailing edge of the primary filaments. The afferent branchial and afferent filamental arteries have relatively low resistances and little pressure is lost by blood passing through them. The blood then flows through the afferent lamellar arterioles, through the lamellae, and passes out through the efferent lamellar arterioles into the efferent filamental arteries. The lamellae and lamellar arterioles have a relatively high resistance and constitute about 68% of the total gill vascular resistance of the lingcod (*Ophiodon elongatus*) (Farrell and Smith, 1981 cited in Satchell, 1991). Not all lamellae are perfused in a resting fish (about $\frac{2}{3}$ in the lingcod at rest (Farrell *et al.*, 1979)) as the afferent arterioles at the distal end of the filaments are closed. These vessels open when blood pressure increases past a critical level, due to exercise or hypoxia for example, which recruits lamellae and increases the functional gas exchange area of the gill. Once open these arterioles remain so below their critical opening pressure (Busby and Burton, 1965 cited in Farrell *et al.*, 1979) which suggests pulse pressure may be more important in opening the arterioles than the average blood pressure. The increased pressure also causes the lamellae to swell, probably flexing the flange-like ends of the pillar cells within the lamellae, rather than stretching the pillar cells (Satchell, 1991). This swelling also increases the functional surface area for gas exchange. Swelling of the lamellae forces a larger proportion of blood through the spaces between the pillar cells rather than through the marginal and apical channels, situated around the edge of the lamellae distal to and within the filament respectively, and exposes a greater proportion of blood to the gas exchange surface of

the lamellae. The efferent lamellar arterioles and efferent filamental arteries may also constrict via parasympathetic innervation to increase the blood pressure within the lamellae.

The entrance to the arterio-venous circulation occurs along the efferent filamental artery in most species of teleosts (Laurent, 1984). When blood pressure increases in this vessel a larger volume of blood is forced into the arterio-venous circulation. This blood is usually needed more urgently elsewhere, as pressure increase generally occurs at times of systemic oxygen debt, so the entrances to the arteriovenous system may constrict via adrenergic innervation or possibly 5-hydroxytryptamine in the blood (Sundin, 1996). From the efferent filamental arteries the blood enters the efferent branchial arteries and then the dorsal aorta. Blood pressure decreases 25 - 50 % across the branchial circulation. Dilation of vessels within the gill will decrease resistance as resistance is proportional to the diameter of the vessels to the fourth power (Randall, 1970). A decrease in branchial resistance could be caused by direct neural or hormonal effects (eg. adrenaline) or vessel stretching due to increased blood pressures (Wood, 1974). Similarly, recruitment of lamellae will also decrease resistance as resistance in parallel is proportional to the sum of the inverse of all individual resistances.

In the present study some of these effects were taken into account when designing the experiment. It has been shown that pulsatile flows, which simulate the condition *in situ* more accurately, enhance the performance of perfused gill preparations (see Perry and Farrell, 1989 for a review) probably by recruiting a larger number of lamellae (Farrell *et al.*, 1979). Even though a pulsatile flow was used in the present study pre-branchial pressure was high and efferent flow was low suggesting branchial resistance was very high. Wood (1974) found that gill resistance increased with time in the perfused head of the rainbow trout and that the increase could not be stopped. However, the increase in resistance could be slowed by using 4 % P.V.P. as a substitute for colloid, irrigating the gills with oxygenated water to prevent hypoxic effects, performing the experiment at low temperatures (5 °C) and millipore filtering the perfusate to prevent clogging of the gill vasculature. None of these precautions were taken in the present study and so oedema of the gill tissue may have occurred due to both the high perfusate pressure and the lack of plasma protein in the perfusate. Also, efferent pressures were quite low (18 mmHg) compared to teleost dorsal aortic pressure values of 22 - 31 mmHg (from Olson, 1998b; Satchell, 1991). It has been shown that efferent pressures are more important than afferent pressures in decreasing branchial resistance and increasing flow through the gills (Wood, 1974). However, significantly more than resting levels of adrenaline were added to the perfusate in an attempt to dilate the branchial vasculature and reduce resistance. Given the distensibility of the branchial vasculature and the supra-physiological perfusate pressure the ability of a vasodilator to show an effect on pressure and/or flow, as phentolamine was expected to have done in the presence of adrenaline, is

uncertain. 5-hydroxytryptamine did show a vasoconstriction so the data suggest none of the three anaesthetics caused vasoconstriction. The anaesthetics were placed in the solution around the gill whereas 5-hydroxytryptamine, which did have an effect, was placed in the perfusate. It is possible that the anaesthetics did not penetrate the gill. However, as fish inhalation anaesthetics are almost exclusively taken up via the gill this is very unlikely. MS222 is very lipid soluble and passes across the gill membrane very rapidly (Adamson, 1967).

The efferent flows recorded by Fromm *et al.* (1971) were in the range of 33 - 41 $\mu\text{L} \cdot \text{min}^{-1}$. If efferent flow was at physiological rates this would suggest that the size of the fish used were about 22 g ($41 \mu\text{L} \cdot \text{min}^{-1} \times \text{eight gills} \times 15 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ cardiac output = 21.8 g assuming the majority of perfusate went through the arterial route). The size of the fish used is not stated but it is unlikely they would be this small. It is therefore likely that their gills were under perfused. The perfusate was pumped into the gills using constant pressure. India ink perfusion showed that 83 - 98 % of the lamellae were being perfused. This suggests low lamellar recruitment was not a problem, although measures of proportion of lamellae perfused were highly variable and did not correlate well to changes in efferent flow rate. Adrenaline was not added to the perfusate (Richards and Fromm, 1969) which could have lowered branchial resistance. Adrenaline is normally present in fish blood at concentrations of between one and five $\text{nmol} \cdot \text{L}^{-1}$ (Randall and Perry, 1992) which should give a mild β -adrenergic vasodilation (Wood, 1974) and reduce resistance. Finally, the statistical analysis used may be overgenerous in its ability to find differences. $P \leq 0.1$ was used as the level of significance which is twice that normally used today. Importantly, the treatment values were compared with pretreatment values, and not the control gill values at the same time, even though the control gills' efferent flow decreased by 10 % (not a statistically significant decrease). Given these methodological problems the decrease in efferent flow caused by 100 ppm MS222 (Fromm *et al.*, 1971), although statistically significant, must be treated with caution.

Overall, the evidence from the perfused gill work in this study and the work of Fromm *et al.* (1971) does not clarify what is occurring in the gill during anaesthesia. The main reason for this is the complex nature of blood flow in the gill and difficulties in controlling this experimentally. A simpler model was required in order to get useful information and it was for this reason that myography was used. Although the data from myography are more limited, in that any changes observed must be integrated into the function of the gill as a whole, it is an excellent technique in that it is relatively easy to accomplish and that real effects are easily discerned from artefact. Also, general changes in vasoactivity can be cautiously applied to vessels other than those in the gill.

The data from the myography experiments showed that all of the anaesthetics had a vasodilator effect on the efferent arteries of the spotty and salmon at one or more of the concentrations tested.

MS222 affected the afferent vessels of both species and metomidate did not affect the afferent vessels of either species. The effect of Aqui-S on salmon vessels was not straightforward as in six out of nine of the efferent vessels and seven out of eight of the afferent vessels the tension at 100 % concentration was greater than the tension at 50 % concentration. This was not seen in the controls which were run at the same time and treated identically. As lower concentrations cause a dilation, and the increase in tension occurs late in the experiment, the effect may be time dependent. The increase in tension was not seen in the spotty vessels, which also tested higher final concentrations of the anaesthetics, but the duration of the spotty myography experiments were 15 minutes shorter than the salmon myography experiments.

For MS222 and Aqui-S the effect on the salmon efferent vessels at 10 % anaesthetic concentration was not increased significantly with higher doses of anaesthetic. This shows these anaesthetics are potent vasodilators. MS222 and Aqui-S may show a dose dependent effect using a range of lower concentrations. In contrast metomidate did have a dose dependent response in the anaesthetic range tested which suggests a maximal response may be seen at higher concentrations. In general MS222 and Aqui-S were more potent vasodilators than metomidate. MS222 and Aqui-S had similar effects on efferent vessels but MS222 had a greater vasodilator effect than Aqui-S on the afferent vessels.

The anaesthetics had a larger effect on the efferent vessels than on the afferent vessels. The afferent vessels contain blood at a higher pressure and are visibly thicker than the efferent vessels. Therefore the afferent vessels may have a greater proportion of relatively inert connective tissue compared to vasoactive smooth muscle. Alternatively, the efferent vessels may contain proportionally more smooth muscle as it is these vessels which have greater control over gill perfusion via contraction and relaxation of this muscle.

Other points to consider are 1) whether or not all of the vessels are equally susceptible to the vasodilating effects of the anaesthetics, and 2) whether the anaesthetized fish lose hormonal and neural control of the vasculature such as the efferent filamental sphincters and the afferent and efferent lamellar arterioles. The first question is partially answered by comparing the myography results from the afferent and efferent arteries. These two vessel groups differed in their reaction to the anaesthetics, so it is possible that certain sections of the afferent and, more importantly, the efferent vessels may show reactivity differences. If vessels or sections of vessels which have a large influence on haemodynamics, such as the efferent and afferent lamellar arterioles, are not affected directly and/or hormonal and neural control of these vessels are resistant to the direct effects of an anaesthetic, then that anaesthetic may have little effect on the gill. It is unknown whether or not hormonal and neural control would be lost due to the presence of anaesthesia.

Humans patients under halothane anaesthesia lose the baroreceptor initiated positive chronotropy in response to a decrease in blood pressure (Stoelting and Miller, 1989). Similar blocking of homeostatic responses in fish could alter the haemodynamics of gills in an unexpected manner. *In vivo* work may answer some of these questions.

If a general vasodilation occurred, what effect would this have upon gill perfusion? Answering this question is for the most part speculation. Firstly, an anaesthetic induced vasodilation in the gill may occur in conjunction with a systemic vasodilation. Systemic vasodilation would reduce dorsal aortic pressure, a regulator of gill haemodynamics, and may affect venous return which would affect cardiac output, and therefore gill perfusion. Also, anaesthesia almost always causes some stress and activity which would occur in conjunction with the anaesthetic effects. As anaesthesia affects respiration, hypoxia may occur which could induce cardiovascular changes.

Chapter 5

General Discussion

WHOLE ANIMAL AND ORGAN STUDIES

Changes to the cardiovascular system can be mediated via endogenous neural, hormonal, local and physical effects and also by exogenous foreign compounds (for examples of cardiovascular control mechanisms see Nilsson, 1984a; Olson, 1998b; Randall, 1982; Randall and Perry, 1992; Satchell, 1991; Taylor, 1992; Wood, 1974; Wood and Shelton, 1975). Both the branchial (Sundin, 1996; Wood, 1974) and systemic circulations (Wood and Shelton, 1975) can be altered by the endogenous factors listed above, but the exact nature of the changes vary between the two circulations because a particular change in the branchial circulation may not be helped, or may even be hindered, by an identical change in the systemic circulation (Wood and Shelton, 1975). Whereas neural, hormonal and local effects can change the systemic and branchial circulations differently, physical changes, such as changes in efferent and afferent pressure or flow, will affect the circulations similarly (Wood and Shelton, 1975). For this reason, when a change in the circulation is observed, explanations due to physical changes can be suggested with more confidence than explanations that include changes due to neural, hormonal and local effects. Exogenous drug effects must be placed in the category with neural, hormonal and local effects because, although the presence or absence of the drug can be established, its mechanism(s) of action may not be known. It is for this reason *in vitro* studies are useful, as they can show direct effects of drugs. However, the presence of an effect *in vitro* does not imply an effect *in vivo* for two reasons. Firstly, in whole animal studies, even if a drug that affected an organ *in vitro* did reach that organ in an adequate concentration *in vivo*, the primary effect of that drug could be compensated for, or abolished by, the endogenous controls mentioned above, such that no effect is observed *in vivo*. Secondly, unless the drug is limited to the organ in question, it might affect other tissues which may cause secondary effects not mediated by the organ under scrutiny. With these considerations in mind an attempt was made at integrating the *in vivo* data from Chapter 2 and the *in vitro* data from Chapter 3 and Chapter 4.

Metomidate

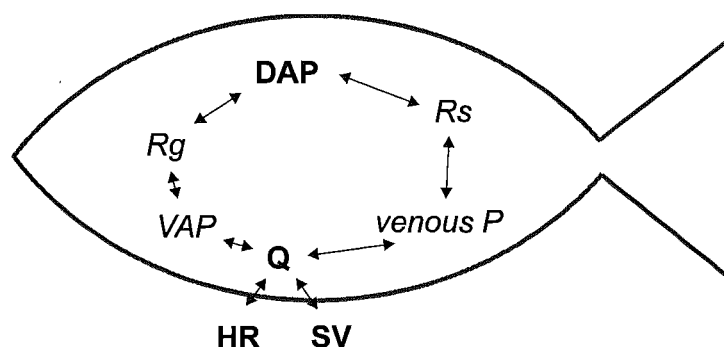
Of the three anaesthetics tested metomidate caused the least side effects, in number and magnitude, and so will be discussed first. During the five minute exposure of whole fish to seven ppm metomidate (Experiment 2.2a) heart rate, cardiac output, dorsal aortic blood pressure and stroke volume were all unaffected. Blood catecholamine levels and haematocrit did not change but dorsal aortic blood PO_2 decreased significantly. *In vitro*, metomidate caused a reduction in the contractile force of ventricle strips (Series 3.1), had an effect on vagal transmission only at very high concentrations (Series 3.2) and caused a dose dependent vasodilation of the efferent branchial arteries (Series 4.2). The afferent branchial arteries were not significantly affected by even high (100 ppm) concentrations of metomidate. These changes are summarised in Figure 5.1.

As there were no changes in the measured cardiovascular variables *in vivo* (Experiment 2.2a) the reduction in PaO_2 probably resulted from non-cardiovascular changes. The most likely cause was a reduction in ventilation amplitude and volume which always accompanies deep anaesthesia (Bell, 1987; Brown, 1988; Iwama *et al.*, 1989; Ryan, 1992a; Ryan, 1992b; Schoettger and Julin, 1967). The rate of decline of PaO_2 will correlate with the speed with which ventilation is impaired by anaesthesia, as transport of oxygen into the blood will be attenuated more quickly but removal of oxygen from the blood by metabolising tissues will continue.

Having suggested reasons for the positive *in vivo* results from Chapter 2, the positive *in vitro* results from Chapters 3 and 4 that were not manifested *in vivo* need to be addressed. Metomidate caused a vasodilation of the efferent branchial arteries. Vasodilation of the branchial vasculature might be expected to cause an increase in dorsal aortic pressure, as resistance through the branchial vasculature to the dorsal aorta may decrease. In contrast, a similar vasodilation in the systemic vasculature might be expected to do the opposite, as reducing resistance downstream (systemic vasculature) will reduce pressure upstream (dorsal aorta). In the whole animal exposed to seven ppm metomidate for five minutes there was no change in dorsal aortic pressure. There are four possible explanations why the observed *in vitro* vasodilation was not evident *in vivo*.

Firstly, the lack of response could be due to an inadequate accumulation of anaesthetic in the tissues. This is unlikely because the branchial vasculature is at the site of anaesthetic acquisition and the systemic vasculature is perfused with blood almost directly from the gill. Also, vasodilation *in vitro* occurred at only 1.75 ppm, four times less than the bath concentration.

decrease in PaO_2 - decrease in ventilation volume



In vitro - caused decrease in ventricular contractility - no effect *in vivo*?
- affected neural transmission down vagus nerve at high concentration
- caused dilation of efferent branchial arteries - no effect *in vivo*?

Figure 5.1. Diagram summarising the *in vivo* and *in vitro* effects of metomidate and the likely causes. Variables in normal text were measured and italicised variables were not measured. See text for details. PaO_2 = the partial pressure of oxygen of the blood in the dorsal aorta, DAP = dorsal aortic blood pressure, *Rs* = systemic resistance, venous P = venous blood pressure, Q = cardiac output, HR = heart rate, SV = stroke volume, VAP = ventral aortic blood pressure, *Rg* = gill or branchial resistance.

A second possibility is that vessels in the *in vivo* experiment did not have time (Experiment 2.2a) to react to the metomidate. The *in vitro* experiment exposure time was 15 minutes (Series 3.2) whereas the total time the branchial and systemic vasculature was exposed to the anaesthetic in the whole fish was less than five minutes, if the time required for adequate anaesthetic to accumulate is taken into account. However, *in vitro* changes in branchial artery vasoactivity occurred almost immediately after addition of metomidate, which suggests the five minute exposure of the whole fish was ample time to see an effect if one was to occur.

A third possibility is that the actions of metomidate are selective and that it does not cause vasodilation of some vessels. The efferent branchial vessels were shown to dilate *in vitro* in the presence of metomidate, but the afferent vessels did not. Therefore, it is not known whether vessels from the systemic vasculature will vasodilate in the presence of metomidate. However, all of the anaesthetics caused a lower vasodilatory response in the afferent branchial arteries compared to the efferent branchial arteries and the reason for this might be due to vessel morphology. It may be that the afferent vessels require a very high concentration of metomidate to cause vasodilation. Afferent branchial arteries are thicker and probably contain more elastin than the efferent branchial arteries and therefore may not stretch as much when under tension (Bushnell *et al.*, 1992; Satchell, 1991). The vessels in the systemic circulation are more like the efferent branchial arteries in that they are thinner, as the blood they carry is at a lower pressure, and are

probably more vasoactive *in vivo*. Also, the systemic vessels are known to be more distensible than the branchial vessels which suggests their ability to relax or stretch is greater (Wood and Shelton, 1975) and that their response to vasoactive compounds will be more similar to efferent branchial arteries than afferent branchial arteries. Another possible difference between types of vessels, which could effect anaesthetic action, is the presence, absence or variation in number of active sites. General anaesthetics are much more selective than is usually appreciated and may act by binding to only a small number of targets (Franks and Lieb, 1994). If possible targets were missing in a particular organ or tissue, then that organ or tissue will not be affected. A lack of suitable active sites will be more likely to affect general anaesthetics and hypnotics, such as metomidate and analogues of Aquil-S, than local anaesthetics, like MS222, which have mechanisms of action that can effect all muscle and nerve types. Separate regions of the fish vasculature are affected differently by the same hormones by possessing different receptors or by lacking receptors. For example, the catecholamines adrenaline and noradrenaline generally cause significant vasodilation in the arterio-arterial branchial vasculature but cause a smaller vasoconstriction in the systemic vasculature (Davie, 1981; Wood and Shelton, 1975) as the two vessel systems possess different α and β -adrenoceptor proportions. Similarly, angiotensin II causes systemic vessels to constrict whereas other vessels are unreactive or relax (Olson, 1998b). Therefore, a variation in receptor distribution between different tissues could explain some of the differences in effects of anaesthetics.

The fourth possibility is that metomidate did cause a vasodilation but that it was negated by an endogenous vasoconstriction caused, for example, neurally or by blood borne hormones. An increase in plasma catecholamines was not observed but other endogenous vasoconstricting compounds may have been released. Nerve conduction was not affected by the presence of metomidate which suggests the normal vasoconstricting tonus would not be affected, at least via nerve blockade. The gill vasculature resistance can be altered by a variety of neural and local effects (see Chapter 4 and Sundin, 1996). However, metomidate is supposed to possess strong central muscle relaxing properties (Hall and Clarke, 1991) which suggests the normal cardiovascular reflex responses could be inhibited. Without direct evidence of the loss of a centrally mediated reflex vasoconstriction the simplest and most adequate explanation is that homeostatic responses nullified any possible effects of exogenous vasoactivity by metomidate.

Metomidate caused a reduction in ventricle contractile force at ten ppm *in vitro* but not at five ppm (Series 3.1). A reduction in ventricular contraction could affect blood pressure and cardiac output in the whole animal (Aitkenhead and Smith, 1996; Black, 1980; Stoelting and Miller, 1989). However, there were no cardiovascular changes measured in whole fish in response to five minutes of seven ppm metomidate exposure (Experiment 2.2a). There are a number of possible

reasons for this. Firstly, it is possible an inadequate amount of metomidate was taken up by the heart, because not only is the heart the last organ to be perfused (metomidate may have been sequestered by previously perfused tissues) but also the concentration of metomidate needed to cause a significant reduction in contractile force was more than five ppm, and possibly up to ten ppm, and the bath concentration was only seven ppm. Secondly, even if there was a significant decrease in ventricular contractility it may not affect cardiovascular parameters in the whole animal. If the maximum power output of the heart is reduced an effect may only be noticed if the heart is required to perform a work load above the new reduced maximum. The heart's potential to increase cardiac output will be reduced, but its ability to maintain a workload lower than the new reduced maximum might be unaffected (see Chapter 3 and Ryan *et al.*, 1993). Therefore, experimental measurement of a decrease in maximum power output, as performed by Ryan *et al.* (1993) on rainbow trout (*Oncorhynchus mykiss*) hearts *in vitro*, may be required to identify a heart with impaired performance.

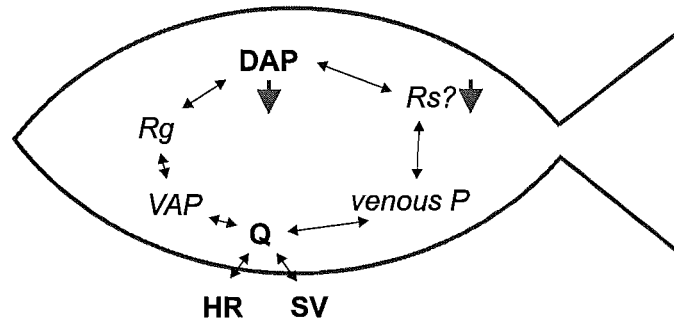
MS222

MS222 had the next greatest effect on whole animals but, in most cases, its *in vitro* effects were greater than or equal to those of Aquí-S. MS222 affected only one of the cardiovascular variables in whole fish (Experiment 2.2a), dorsal aortic pressure, which progressively decreased with increased exposure time. Haematological changes were identical to metomidate, with a decrease in PaO₂, but with no change in plasma catecholamine levels or haematocrit. MS222 caused greater *in vitro* branchial artery vasodilation than metomidate (Series 4.2), a greater reduction in ventricular contractility than metomidate and Aquí-S (Series 3.1) and attenuated conduction along the vagus nerve, which neither of the other anaesthetics did at average anaesthetic bath concentrations (Series 3.2). These results are summarised in Figure 5.2.

The reduced PaO₂ in MS222 treated fish was similar in magnitude to that of the metomidate treated fish, and animals from both treatments showed a lack of activity during the exposure. This suggests the drop in PaO₂ in both treatments had the same primary cause, which is likely to be a reduction in ventilation volume.

The progressive decrease in dorsal aortic pressure could have a single cause or a combination of causes. There are three main cardiovascular changes that could have decreased dorsal aortic pressure: 1) a decrease in contractile force of the heart, 2) an increase in branchial resistance and 3) a decrease in systemic resistance. A decrease in myocardial contractile force will decrease the maximum power output of the heart. If this is severe enough then providing the work required to

decrease in PaO_2 - decrease in ventilation volume



- In vitro* - caused decrease in ventricular contractility - no effect *in vivo*?
- caused decrease in neural transmission down vagus nerve - effect *in vivo*?
- caused dilation of efferent and afferent branchial arteries - effect *in vivo*?

Figure 5.2. Diagram summarising the *in vivo* and *in vitro* effects of MS222 and the likely causes. Variables in normal text were measured and italicised variables were not measured. Black arrows show measured effects and grey arrows show likely effects. PaO_2 = the partial pressure of oxygen of the blood in the dorsal aorta, DAP = dorsal aortic blood pressure, R_s = systemic resistance, venous P = venous blood pressure, Q = cardiac output, HR = heart rate, SV = stroke volume, VAP = ventral aortic blood pressure, R_g = gill or branchial resistance. See text for details.

move an adequate blood flow around the body under normal circumstances may become difficult. However, MS222 treated fish maintained cardiac output. No change in heart rate or stroke volume was observed which suggests the heart was probably functioning adequately.

An increase in branchial resistance could cause a decrease in dorsal aortic pressure and, concurrently, an increase in ventral aortic pressure. There were no obvious signs of the heart being challenged by an increased afterload, such as a decrease in stroke volume, so there is no evidence that an increase in branchial resistance occurred. MS222 caused the branchial arteries to dilate *in vitro* which suggests that if MS222 had a direct effect on the branchial vasculature it would be a dilation and would cause a decrease in branchial resistance. This would cause dorsal aortic pressure to increase unless systemic resistance decreased to a greater extent, in which case venous return might increase and cardiac output would increase in turn. Central or peripheral interference by MS222 of a neural tonus to the gill vasculature (Wood, 1974) would also result in a vasodilation. Therefore an increase in branchial resistance would have to be caused by endogenous effects. Plasma catecholamines, which can also cause branchial vasodilation (Nilsson, 1984b; Sundin, 1996; Wood, 1974), did not increase (Experiment 2.2a), but locally released chemicals such as 5-hydroxytryptamine (alternative name: serotonin) or adenosine could cause a branchial vasoconstriction. 5-hydroxytryptamine increases perfusion pressure in the perfused gill arch of the Atlantic cod (*Gadus morhua*) (Sundin and Nilsson, 1992). 5-hydroxytryptamine is released from neuroepithelial cells during, and some of its responses mimic, severe hypoxic and

acidotic events in fish (Sundin *et al.*, 1995). Adenosine, also a branchial vasoconstrictor, is released from energy deficient cells due to the breakdown of adenosine phosphates (Sundin, 1996). However, both 5-hydroxytryptamine and adenosine are released during severe hypoxia (Sundin, 1996) so their release would have been expected in both the MS222 and metomidate treated fish as these treatments caused the same amount of hypoxia. Overall, there is no solid evidence for or against an increase in branchial resistance, but it is difficult to conceive how it could occur.

The third possible way in which dorsal aortic pressure could decrease is via a decrease in systemic resistance. There is some circumstantial evidence for this. In the MS222 treated fish, cardiac output, stroke volume and heart rate do not change. If it is assumed that any change in venous pressure (normally about one to three cmH₂O, can range from -5 - 13 cmH₂O, (see Randall, 1970; Satchell, 1991)) is lower than the change in dorsal aortic pressure (about ten cmH₂O in the MS222 treated fish) then the pressure differential between blood in the dorsal aorta and venous blood ($\Delta P = DAP - \text{venous } P$) must decrease. Cardiac output is constant and should reflect venous return apart from a possible change between arterio-arterial and arterio-venous flow at the gill. If these assumptions are correct then:-

Systemic resistance equals:

$$R_s = \frac{(DAP - \text{venous } P)}{Q}$$

Under normal circumstances relative dorsal aortic pressure, cardiac output and systemic resistance equal one, and venous return pressure equals zero:

$$R_s = \frac{(1 - 0)}{1} = 1$$

If the change in dorsal aortic pressure (ten cmH₂O or about 25 % in MS222 treated fish) is assumed to be greater than any change in venous pressure, and cardiac output does not change then systemic resistance has fallen.

$$R_s = \frac{(\frac{3}{4} - 0)}{1} = \frac{3}{4}$$

A decrease in systemic resistance is possible in two ways. Firstly, MS222 caused both the efferent and afferent branchial arteries to dilate *in vitro*. However, metomidate also caused a vasodilation *in vitro*, although only in the efferent branchial arteries and to a lesser degree, and metomidate treated whole fish did not show a decrease in dorsal aortic pressure. Also the branchial circulation showed no evidence of vasodilation which would increase dorsal aortic pressure if cardiac output was unchanged. The second possible mechanism is by interfering with the neural tonus of the systemic vasculature. Immediately after the initial set up, the resistance through perfused trunks of rainbow trout (*Oncorhynchus mykiss*) (Wood and Shelton, 1975) and short finned eels (*Anguilla*

australis schmidtii) (Davie, 1981) decreased with time. The vasculature of the rainbow trout perfused trunks decreased to a stable resistance below that of the branchial resistance. However, the gills *in vivo* have a lower resistance than the trunk because the pressure differential across the branchial vasculature is lower than that across the systemic vasculature. The decrease in resistance of the perfused trunks was thought to be due to a decrease in neural and/or hormonal α -adrenergic vasoconstricting tonus (Wood and Shelton, 1975). Treatment of rainbow trout with yohimbine, an α -adrenergic receptor blocking agent, prior to set up removed the initial high resistance. Davie (1981) also found a β -adrenergic receptor mediated vasodilation in the short finned eel systemic vasculature, although much smaller in magnitude than the α -adrenergic vasoconstricting tonus. This suggests that if the, probably neural, α -adrenergic vasoconstricting tonus was blocked then systemic resistance would fall, followed by dorsal aortic pressure. MS222 attenuated vagal nerve conduction which suggests it could affect transmission of signals to and from the central nervous system or even directly affect the central nervous system. It has been suggested that MS222 exerts a depressive influence upon central autonomic functions (Houston and Woods, 1972). If the tonus maintaining normal vessel tension was attenuated or blocked in the MS222 treated fish, via either central effects or nerve blockade, then systemic vasodilation caused by a reduced tonus, possibly enhanced by direct anaesthetic induced vasodilation, could occur, leading to a reduction in dorsal aortic pressure. Wood and Shelton (1975) used MS222 to anaesthetise fish prior to preparation of the perfused trunks and found that MS222 had no significant effect on reducing the initial high systemic pressure. However, when these measurements were made the spinal cord had been transected and the trunk perfused with saline for five to ten minutes which suggests neural control could have been lost and/or that MS222 could have been washed away. There remains some doubt as to the ability of the experiment of Wood and Shelton (1975) to show a central or peripheral block of the α -adrenergic vasoconstricting tonus in the systemic vasculature by MS222. The fact that MS222 attenuated transmission down the vagus nerve *in vitro* (Series 3.2) suggests that the α -adrenergic vasoconstrictor tonus could have been blocked peripherally as opposed to centrally. In contrast, Lochowitz *et al.* (1974) showed clearly that the ability of the vagus to reduce heart rate was not abolished in rainbow trout (*Oncorhynchus mykiss*) anaesthetised with MS222.

A reduction in dorsal aortic pressure, which occurred in the MS222 treated fish (Experiment 2.2a), can increase branchial resistance by allowing distensible vessels to relax and partially collapse (Wood, 1974). Also, reduced efferent branchial pressure might reduce intralamellar blood pressure which could cause a decrease in the number of lamellae perfused and reduce the perfusion of individual lamellae by allowing blood to move predominantly through the marginal and apical channels of the lamellae and not between the pillar cells. This could reduce the efficiency of oxygen extraction and reduce PaO_2 . A reduction in dorsal aortic pressure was not observed in metomidate

treated fish, and the PaO₂ of metomidate and MS222 treated fish were similar, which argues against systemic vasodilation being a major factor influencing gas exchange at the gill. It was suggested above that the predominant reason PaO₂ declined in MS222 and metomidate treated fish was a reduced ventilation volume limiting oxygen uptake, which still seems the most likely cause.

MS222 caused a greater decrease in ventricular contractility *in vitro* than metomidate yet neither anaesthetic had any effect on cardiac output in whole fish. The possible reasons for this lack of effect were discussed above with respect to metomidate. The amount of metomidate required to affect ventricular contractility was high (five to ten ppm) compared to the bath concentration (seven to ten ppm). In contrast, only ten ppm of MS222 was required to have an effect on ventricle strip contractility *in vivo* (Series 3.1) which is 10 % of the normal bath concentration, so a lack of effect due to a lack of MS222 in the ventricle is unlikely. This again suggests that the work rate of the hearts of the whole fish was below any hypothetical reduced maximum due to the negative inotropic effects of the anaesthetics and also supports the idea that the decrease in dorsal aortic pressure was not due to a compromised heart. Figure 5.2 summarises the likely reasons for the changes caused by MS222.

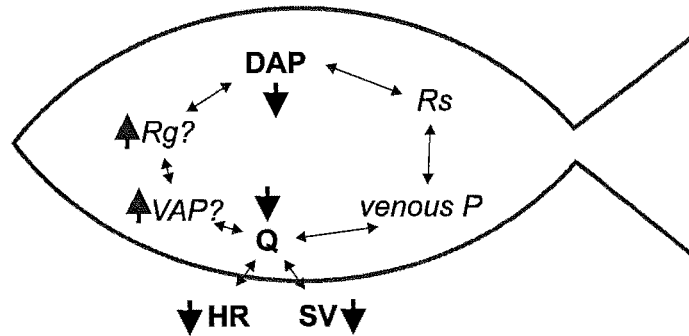
Aqui-S

Exposure to Aqui-S caused an initial reduction in heart rate, cardiac output, dorsal aortic blood pressure and stroke volume as well as initiation of violent activity in whole fish (Experiment 2.2a). These changes are summarised in Figure 5.3a. Heart rate, cardiac output and stroke volume recovered to pre-exposure levels and cardiac output and stroke volume eventually exceeded initial values. Dorsal aortic pressure plateaued after the initial decrease. Dorsal aortic blood PO₂ decreased during the exposure, significantly more so than in MS222 treated fish. Haematocrit and plasma catecholamines both increased during the exposure. Aqui-S caused a small decrease in ventricular contractility (Series 3.1), very similar to that of metomidate, and had a large vasodilating effect on the branchial arteries (Series 4.2), similar to that of MS222. However, the afferent branchial arteries showed more vasodilation to 30 ppm Aqui-S than to 60 ppm which differs from the trend shown by all other treatments. Aqui-S, like metomidate, had little effect on the ability of the vagus nerve to affect the *in situ* perfused heart (Series 3.2). These results are summarised in Figure 5.3b.

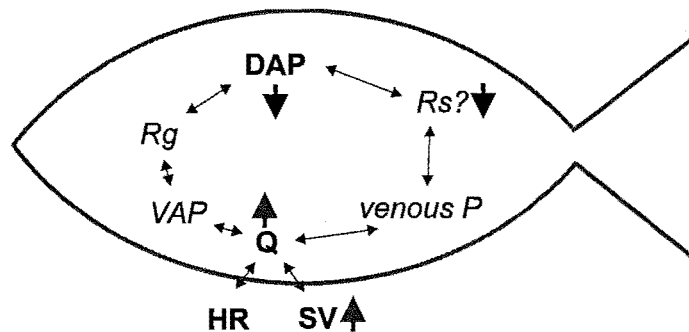
The initial decrease in the four measured cardiovascular variables in whole fish exposed to 60 ppm Aqui-S was most likely due to an alarm response. The active ingredient of Aqui-S is extracted from

decrease in PaO_2 due to - decrease in ventilation volume
 - increase in activity
 increase in plasma catecholamines due to very low PaO_2
 increase in haematocrit due to catecholamine release

a



b



In vitro - caused decrease in ventricular contractility - no effect *in vivo*?
 - affected neural transmission down vagus nerve at high concentration
 - caused dilation of efferent and afferent branchial arteries - effect *in vivo*?

Figures 5.3a&b. Diagram summarising the *in vivo* and *in vitro* effects of Aqu-S and the likely causes early in the exposure (a) and after five minutes exposure (b). Variables in normal text were measured and italicised variables were not measured. Black arrows show measured effects and grey arrows show likely effects. PaO_2 = the partial pressure of oxygen of the blood in the dorsal aorta, DAP = dorsal aortic blood pressure, R_s = systemic resistance, venous P = venous blood pressure, Q = cardiac output, HR = heart rate, SV = stroke volume, VAP = ventral aortic blood pressure, R_g = gill or branchial resistance. See text for details.

cloves, and cloves are known to cause a hot sensation on the tongue in humans. Fish may experience something similar over some or all of their epidermis triggering an alarm response. The physiological cause of the decrease could be a large vagal bradycardia. However, a bradycardia would not be expected to reduce stroke volume, as stroke volume would probably increase due to the Starling effect and the vigorous activity augmenting venous return. Therefore, a branchial vasoconstriction may also have occurred which might increase ventral aortic pressure, if cardiac output did not change, and decrease dorsal aortic pressure. If the branchial vasoconstriction was strong enough then stroke volume could decrease as a result. This reaction was suggested to be a nociceptor mediated response (see Chapter 2) the proposed aim of which is to decrease the

amount of toxin uptake, by reducing flow over the gills via reduction of movement and ventilation, and to interrupt transport of the toxin from the gills to the tissues via bradycardia, which would reduce cardiac output (Satchell, 1991). All variables except dorsal aortic pressure recovered to pre-treatment levels within two to three minutes. Recovery of these variables would be expected when the fish could no longer perceive the sensation that caused the nociceptor response. In this case the loss of sensation was most likely effected via anaesthesia. These conclusions are summarised in Figure 5.3a.

As mentioned above, dorsal aortic pressure did not return to pre-exposure values in Aqual-S treated fish. In addition cardiac output increased. Using the same argument as for MS222:-

Systemic resistance equals:

$$R_s = \frac{(DAP - \text{venous}P)}{Q}$$

Under normal circumstances relative dorsal aortic pressure, cardiac output and systemic resistance equal one, and venous return pressure equals zero:

$$R_s = \frac{(1 - 0)}{1} = 1$$

If the change in dorsal aortic pressure (12 cmH₂O or about 25 % in Aqual-S treated fish) is assumed to be greater than any change in venous pressure, and that cardiac output (which increased by 1.6 times) and venous return remain relative then systemic resistance must fall, more so than for MS222.

$$R_s = \frac{(\frac{3}{4} - 0)}{1.6} \approx \frac{1}{2}$$

A comparison of the *in vitro* and whole animal effects of Aqual-S with metomidate and MS222 may clarify this observation. Metomidate, like Aqual-S, did not affect the vagus nerve at anaesthetic bath concentrations and, also like Aqual-S, caused a vasodilation of the efferent branchial arteries, although Aqual-S was the more effective vasodilator. If the drop in dorsal aortic pressure was caused only by vasodilation as a result of a direct effect of the anaesthetic, then a moderate decrease in dorsal aortic pressure would also be expected with metomidate exposure. This was not the case which suggests vasodilation via direct anaesthetic action is unlikely to cause a drop in dorsal aortic pressure in the whole animal. It was suggested that MS222 caused the decrease in dorsal aortic pressure by attenuating central control of the normal vasoconstricting tonus. This would have allowed the direct vasodilating effect of MS222 to proceed unimpeded by a homeostatic vasoconstriction. Although Aqual-S did not attenuate transmission down the vagus nerve *in vivo*, the results could suggest that Aqual-S affected fish centrally. Central effects of fish anaesthetics have not been studied in any detail so any suggestions of central effects are speculative. However, analogues of the active component of Aqual-S have been used as

intravenous anaesthetics in humans (Aitkenhead and Smith, 1996; Dundee and Clarke, 1979; McDonald and Wann, 1978) and therefore must have central effects. The different effects of metomidate and Aqui-S on whole fish in the present study show that there are obvious differences in either their mechanism of action or side effects. As mentioned in Chapter 3, available information on intravenous hypnotics and anaesthetics suggests that the analogues of the active component of Aqui-S have much greater cardiovascular side effects than metomidate and its analogues (Aitkenhead and Smith, 1996; Dundee and Clarke, 1979; Fischer and Marquort, 1977; Gooding and Corssen, 1976; Gooding and Corssen, 1977; Hall and Clarke, 1991; Reneman and Janssen, 1977; Stoelting and Miller, 1989). Vasoactivity can also occur as a consequence of autoregulation. Vasoactive substances, such as carbon dioxide, lactic acid, adenosine and hydrogen ions, can be released during exercise or hypoxia and cause vasodilation in the peripheral vasculature (Guyton, 1986). This may have contributed to the decrease in dorsal aortic pressure, or the maintenance of a low dorsal aortic pressure, in the Aqui-S treated fish.

After about 3.5 minutes of exposure to Aqui-S an increase in stroke volume (and possibly heart rate) caused an increase in cardiac output in the Aqui-S treated whole fish (Experiment 2.2a). Adrenaline and noradrenaline were released during the exposure and these catecholamines possess the ability to effect positive chronotropic and inotropic changes in the heart (Farrell, 1992). The PaO_2 of the Aqui-S treated fish was very low after the five minute exposure, and significantly lower than that of MS222 treated fish. Aqui-S treated fish also had elevated haematocrit and plasma catecholamine levels. The very low PaO_2 was probably caused by a reduction in ventilation volume, as in the MS222 and metomidate treated fish, in conjunction with the violent activity. The activity was almost certainly anaerobic and as ventilation declined so did the ability to "pay off" any oxygen debt. In this particular case, activity increased the demand for oxygen and anaesthesia decreased the ability to extract oxygen. The low PaO_2 could well have induced the release of catecholamines via a direct effect of hypoxaemia on the head kidney chromaffin tissue (Randall and Perry, 1992; Thomas and Perry, 1992). The increase in haematocrit could be explained by humoral adrenergic stimulation of the spleen as the spleen expels erythrocytes at low plasma catecholamine levels (see Randall and Perry, 1992) leading to erythrocyte recruitment and adrenergic or acid stimulation of red cell swelling. An increase in the haematocrit of snapper (*Pagrus auratus*) anaesthetised with MS222 was found to be due to an increase in both cell volume and cell numbers (Ryan, 1992b). Blood acidification could be respiratory, due to reduced ventilation leading to carbon dioxide build up, or metabolic, caused by accumulation in the blood of acid equivalents from anaerobic metabolites from the violent activity (Satchell, 1991). Blood pH, mean cell haemoglobin concentration and cell counts were not performed in this study, so the exact nature and cause(s) of the increase in haematocrit are unknown.

Again, the *in vitro* reduction of ventricular contractility did not seem to impair cardiac output or stroke volume in the whole animal. The stroke volume and cardiac output of the Aqui-S treated fish even increased late in the exposure. These conclusions are summarised in Figure 5.2b.

Summary of effects and their causes

The observations and explanations suggest there are three types of anaesthetic induced effects. Firstly, there are the direct effects of the anaesthetics. These include all of the *in vitro* effects such as the reduction in branchial artery wall tension, the decrease in ventricular contraction and the attenuation of conduction down the vagus nerve caused by MS222. It is difficult to definitively attribute any changes in the whole fish to any of these *in vitro* effects, even when considered in conjunction with the data from the whole fish studies. Secondly, there are the indirect effects of anaesthesia. These include the decrease in PaO₂ due to reduced ventilation, possibly some or all of the reduction in dorsal aortic pressure in the MS222 treated fish and, similarly, possibly the maintenance of the low dorsal aortic pressure in the Aqui-S treated fish. Lastly, there are the tertiary effects caused by other pharmacological properties of the anaesthetics. In particular is the alarm response observed in the Aqui-S treated fish which affected all of the cardiovascular variables and caused the vigorous activity of the Aqui-S treated fish. This in turn may have intensified the decrease in blood PaO₂. Catecholamine release was probably caused by the very low PaO₂, and the secondary increases in stroke volume and heart rate and the increase in haematocrit were probably caused by the release of catecholamines into the blood. In addition to the three anaesthetic effects are human effects. In this study they consisted of the handling in Experiment 2.2b and the surgery in Experiment 2.2d. These human effects generally caused greater cardiovascular changes than the anaesthetics. The four types of changes are summarised in Table 5.1.

STRESSED INDUCTION

The effects of anaesthesia following handling on heart rate, cardiac output and stroke volume were remarkably consistent regardless of the anaesthetic used. On initiation of recordings at 1.5 minutes post-exposure, the time required for the fish to become quiescent enough to begin recording, heart rate and/or stroke volume were elevated resulting in an increased cardiac output. Heart rate declined with time reducing cardiac output. These exposures lasted for 15 minutes and, although by the end of the exposures heart rates were low, cardiac output was mostly maintained due to the relatively high stroke volumes. The maintenance of high stroke volumes again suggest that the

Table 5.1. The four types of anaesthetic effects.

- 1) Primary (Direct) effects
 - i) reduction in branchial artery tension *in vitro* (all anaesthetics, effect *in vivo* ?)
 - ii) decreased force of ventricle contraction *in vitro* (all anaesthetics, no effect seen *in vivo*)
 - iii) increased axonal stimulation threshold and/or decreased action potential strength (MS222 only, effect *in vivo* ?)
- 2) Secondary (Indirect) effects
 - i) reduced blood PaO₂ due to reduced ventilation efficiency and possibly impaired branchial blood flow (all anaesthetics)
 - ii) DAP fall possibly due to possible systemic vasodilation in conjunction with a loss of tonus caused by either central effects or blocking of conducting nerves (MS222, possibly Aqui-S)
- 3) Tertiary (Alarm response) effects (Aqui-S)
 - i) vagal parasympathetic tonus causing:-
 - decrease in HR leading to decrease in Q
 - branchial vasoconstriction increasing branchial resistance causing:-
 - increase in VAP leading to a decrease in SV (and Q)
 - decrease in DAP
 - ii) very low blood PaO₂ caused by an increase in activity and oxygen consumption in addition to impaired ventilation
 - iii) catecholamine release (due to stress of induction or low PO₂)
 - causing an increase in SV (and possibly HR) leading to an increase in Q
 - iv) metabolic vasodilation
 - metabolites (eg. H⁺, lactate) released into the vasculature during the frantic movement may have contributed to the decrease in DAP
- 4) Human effects
 - i) elevated HR, SV and Q during induction due to handling
 - ii) elevated SV and Q and variable HR during recovery after anaesthesia and surgery

negative inotropic effects of all of the anaesthetics on the ventricle strips (Series 3.1) had little influence on whole fish. The initial increase in heart rate and stroke volume was almost certainly due to the handling prior to anaesthesia and may be mediated via humoral (Randall and Perry, 1992) and/or neural adrenergic stimulation (Saito and Tenma, 1976; Taylor, 1992) and change in cholinergic vagal tone (Nilsson, 1984a). The subsequent decline in heart rate during anaesthetic induction is probably due initially to a decline in adrenergic tonus, as the stimulus causing the adrenergic response is no longer perceived. An additional mechanism must then take the heart rate to below pre-exposure levels as simply removing the adrenergic stimulus could not achieve this. A later effect could be an increase in vagal tonus due to hypoxia (Butler *et al.*, 1977; Farrell, 1982; Fritsche and Nilsson, 1990; Randall, 1982), a response which was shown to be present in rainbow trout (*Oncorhynchus mykiss*) under acute MS222 anaesthesia (Lochowicz *et al.*, 1974). However, quite severe hypoxaemia in the fish exposed to an unstressed induction (Experiment 2.2a) did not cause a decrease in heart rate. The exposure duration of the unstressed fish was shorter and there was no increase in activity in the MS222 or metomidate treated fish, compared to the obvious

increase in activity of the Aqui-S treated fish, which suggests quite severe hypoxia is necessary to cause such a reaction. Another possibility is that the hypoxaemia had a direct effect on the heart which caused a decline in heart rate. Lochowitz *et al.* (1974) found that after 30 minutes of MS222 exposure atropine, which should reverse a vagal bradycardia, could not elevate the depressed heart rate of rainbow trout (*Oncorhynchus mykiss*). If the compact myocardium is starved of oxygen the repolarisation of the ventricle may be slow leading to an increased refractory period and a slower heart rate (see Discussion, Chapter 3). Serfaty *et al.* (1959) found that carp (*Cyprinus carpio*) under prolonged MS222 anaesthesia eventually showed atrioventricular dissociation which the authors attributed to hypoxia.

RECOVERY FROM ANAESTHESIA

Recovery from all of the anaesthetics was notable for an increased dorsal aortic pressure for up to 15 minutes post-anaesthesia. The cause of this is unclear, as no other variables were affected, but may be due to the oxygen debt acquired during anaesthesia. Rainbow trout (*Oncorhynchus mykiss*) anaesthetised with either 30 ppm benzocaine hydrochloride, one ppm etomidate or 275 ppm 2-phenoxyethanol showed an increase in dorsal and ventral aortic pressure after 30 minutes anaesthesia whereas those anaesthetised with 51 ppm MS222 did not (Fredricks *et al.*, 1993). In general, the anaesthetics which had the greatest effects during anaesthesia (etomidate and 2-phenoxyethanol) also caused the largest increases in dorsal and ventral aortic blood pressures during recovery. The authors assumed hypoxia was more severe in the etomidate and 2-phenoxyethanol treated fish which suggested the elevated blood pressures could occur as a result of hypoxia. An elevation in ventral and, to a lesser extent, dorsal aortic blood pressures were seen in rainbow trout when exposed to oxygenated water after being held in hypoxic water (PO₂ down to 20 mmHg) (Holeton and Randall, 1967). This suggests an increase in systemic resistance (Fritsche and Nilsson, 1989; Holeton and Randall, 1967), probably effected via adrenergic vasomotor fibres (Fritsche and Nilsson, 1989), caused the increase in dorsal and ventral aortic blood pressures. Aqui-S treated fish also showed a slightly increased stroke volume at 30 minutes. This may be due to the deeper hypoxia to which these fish were subject, due to the increase in activity and the irritant nature of Aqui-S. All fish showed an increased cardiac output and decreased dorsal aortic pressure at six hours. It is not known why this occurred and the information gained from the *in vitro* experiments does not help elucidate this recovery data. Possibly fish at this time were more sensitive to experimenter disturbance after three continuous hours without contact. Recovery from metomidate anaesthesia may be extremely violent in horses (Hall and Clarke, 1991) and restlessness and delirium may accompany recovery from etomidate in humans (Aitkenhead

and Smith, 1996). Similar phenomena may occur in fish recovering from these and other fish anaesthetics, and could contribute to cardiovascular changes during recovery.

RECOVERY FROM ANAESTHESIA AND SURGERY

Changes in the measured cardiovascular variables during recovery from anaesthesia and surgery were far greater than during recovery from anaesthesia alone. Although there were differences between anaesthetics it was the similarities that were most obvious. All fish initially exhibited a mildly decreased heart rate that lasted for less than one hour followed by a slightly increased heart rate for up to about six hours. Cardiac output was elevated for up to three hours and stroke volume was elevated for up to one hour. The recovery from anaesthesia results are quite different from the results of the recovery from anaesthesia and surgery which suggests that these changes are caused by the surgery and prolonged anaesthesia and that the pharmacological effects of the individual anaesthetics were comparatively mild. The initial low heart rate and high stroke volume strongly suggests vagal inhibition of heart rate and a Starling effect (Satchell, 1991). The later increase in heart rate and decrease in stroke volume could be due to a decrease in the vagal tonus. It should be noted that these increases and decreases in heart rate are not significantly different from pre-anaesthesia values, and are only significantly different from other times (as time was a variable). Cardiac output remains elevated for longer than stroke volume although this may reflect the weakness of the statistical analysis for seeing smaller increases in stroke volume and heart rate, rather than any real effect.

WHICH ANAESTHETIC IS BEST?

After completing a study such as this a question which should be answerable is "Which anaesthetic is best?" An objective response is "What do you want it for?" There are a large number of fish anaesthetics available and each has its own strengths and weaknesses. There is no anaesthetic which will suit all tasks. The major use of fish anaesthetics is increasing manageability either by sedating or anaesthetising the fish. The next most important task would probably be surgery, either experimental or veterinary. The ability of the anaesthetics examined in this study to fulfil these tasks will be discussed in the next sections.

Manageability

A survey of American and Canadian fish hatcheries was performed in 1983 to find out which anaesthetics they were using, their perceived limitations, and what they would like to see in a new anaesthetic (Marking and Meyer, 1985). Most (79 %) responders used MS222 which is not surprising as MS222 is the only fish anaesthetic, apart from carbon dioxide, which can be used on food fish. Interestingly, 22 % of responders used quinaldine or quinaldine sulphate which are not registered but which are cheaper than MS222. Only 2.2 % used etomidate and none used metomidate. The biggest complaint about MS222 was its 21 day withdrawal period followed by the fact that it was expensive. The three properties most requested in a new anaesthetic were for it to induce anaesthesia within three minutes, for recovery to occur within ten minutes and for no mortality to occur within 15 minutes. Sixteen chemicals were tested on rainbow trout (*Oncorhynchus mykiss*) for these properties and only four met the requirements; MS222, quinaldine sulphate, benzocaine and 2-phenoxyethanol (Gilderhus, 1989). Metomidate was rejected because its recovery time was almost twice that sought. Although not tested quantitatively in this study, Aqui-S also seems to have a relatively long recovery period. Quantitative studies using clove oil on rainbow trout showed that recovery took six to ten times longer than with MS222 (Keene *et al.*, 1998). Also, the suggested concentration at which Aqui-S should be used is 17 ppm with a corresponding induction time of about 12 - 15 minutes (Aqui-S users manual). Bell (1987) suggested a number of properties for "an ideal anaesthetic" (see Table 1.2). All three anaesthetics tested in this study met most of these requirements. Metomidate and Aqui-S have a long recovery time, and Aqui-S may also fail because one criterion is that the anaesthetic be non-irritant. However, Aqui-S has a very large advantage in that it is made up of edible products and therefore should have little problem in being allowed to become a food fish anaesthetic.

Of the three anaesthetics examined in this study there is no clear favourite. The fast recovery time of MS222 is a definite advantage. However, the edibility of Aqui-S for use as a food fish anaesthetic may be a necessity depending on where the fish are to be sold. Lastly, the cortisol blocking effect of metomidate may be advantageous long term as it may remove the potentially detrimental effects of cortisol, such as reduced growth and impaired immune system (Schreck, 1993), in fish that are frequently disturbed.

Surgery

MS222 is a good anaesthetic for fish surgery because of its relatively rapid recovery rate and its known analgesic effects. However, as with all anaesthetics it reduces the respiration rate making

the subject fish susceptible to hypoxia. This study showed that MS222 has some potentially detrimental cardiovascular side effects, however, it has been used successfully on many occasions, so the detrimental effects cannot be too severe.

Metomidate's main drawback is that it may not have any analgesic effect in fish as it does not produce analgesia in mammals (Hall and Clarke, 1991) and therefore should not be used for surgery (Brown, 1988). Fish anaesthetised with metomidate are prone to spontaneous activity (Stoskopf and Arnold, 1985), and physical manipulation can also elicit movement (Hall and Clarke, 1991). Also the occurrence of spontaneous activity can make it difficult to estimate the depth of anaesthesia (pers. obs.) as loss of movement and the loss of the ability to react to stimuli are important cues in establishing depth of anaesthesia.

Aqui-S caused the greatest cardiovascular side effects. Of more importance is the irritant nature of the mixture and the detrimental effect this could have on a fish undergoing surgery. However, the effects of anaesthesia in combination with other manipulations, such as surgery, tended to hide the inherent effects of each anaesthetic. Therefore, although the direct effects of anaesthetics cannot be altered, secondary anaesthetic effects, such as hypoxia from impaired ventilation or handling stress, which can have a greater effect on fish physiology than the anaesthetic, can be at least partially controlled. Therefore, gentle treatment prior to anaesthetisation and during recovery is recommended. Similarly, if the handling of fish prior to anaesthesia or the anaesthetisation process is not gentle then selection of an anaesthetic with minimal cardiovascular side effects is unnecessary.

MS222 still seems to be the best anaesthetic for surgery. Aqui-S is workable but its longer recovery time and possible irritant effects are not very satisfactory for surgery. As mentioned above, metomidate should not be used for invasive procedures unless absolutely necessary because of its possible absence of analgesic properties.

Acknowledgements

I owe a great deal to many people for helping me get to this stage. A big thank you to all of the following.

Thank you and lots of love to my family, Mum, Dad and Stephen (& Julie), for giving me the best start and always supporting me in whatever I attempted. Also, life over the last 10 years (yes, 10!!) of academia would not have been nearly as good without the joy of the company of Sam, Merry, George and Bludger.

To my supervisor Assoc. Prof. Malcolm Forster. Many thanks for sharing your enthusiasm of the subject and your enjoyment of practical work, for always making time for my questions and for not getting too annoyed when my effort was less than satisfactory.

To my second supervisor (and supervisor of my MSc.) Assoc. Prof. Bill Davison. Thanks for putting up with me for so long, making time for questions and proofreading, while coping with the administrative nightmare of being HOD. Also, a big thank you for the opportunity of a trip of a lifetime.

Thanks to Alastair Jerrett and Crop and Food Research for the monetary support that allowed me to pursue this project and, of course, the Aqui-S.

Thank you to Chris Glover for gathering the myography data on the salmon branchial vessels and to Dr William Titulaer for performing the oxygen blood equilibrium work.

Thanks to Dr Steven Gieseg and Assoc. Prof. Larry Field for advice on the HPLC and nerve work respectively.

A big thank you to Mike Field-Dodgesun and Isaac's Salmon farm for providing many of the fish for this study.

Without the help of a number of technicians much of the data in this project would have taken much longer to obtain or would not have been gained at all. For their efforts I would especially like to thank Gavin Robinson, Victor Mencil and Nick Etheridge. For making life much easier with regards to administration and obtaining material I would like to thank Tracy Robinson, Helen de Groot and Bruce Lingard. Thank you also to Dave Tattle and Rennie Bishop for obtaining some of the animals, and to Terry Williams for his photographic work. Also, thanks to Jan Mackenzie for helping with lots of little and not so little tasks, especially with the poster for the 1996 Australian and New Zealand Society for Physiology and Biochemistry 12th annual meeting.

Thanks to Prof. Ken Olsen for a number of useful discussions and advice.

A big thank you to Mike Hickford for your help with the nightmare of statistics. You helped me get to the stage where I almost thought I knew what I was talking about - at least I could convince some others that I did. Also thanks for the use of "the machines" for the seminar and for the final printing.

Thanks to Craig Morley for putting up with me in the same room and being a good friend for 4 years.

Thanks to Andrew "Roo" Holyoake, the "old hand" at being a PhD. student when I started, for your help and friendship. Also, extreme gratitude for the vast amount of proof reading at the end, totally beyond the call of duty, and very much appreciated.

Thanks to assorted post-grads for late night coffees and chats including, the Queen of late night coffees and chats, Abigail Ball, and also Sue Richards, Olaf Kleisse and Caroline Bunn. Also thanks to Abs for being a good friend.

I appreciated the company of, and discussions with, my fellow fish phys. people; Anna Herriot, Chris Glover, Charles Simpson and Suzie Black.

Varsity at times was made bearable by persons outside the confines of the campus. Very big thank you's to Mark, Anne and Catherine for being good friends and for always being kind and generous when I "just popped around" uninvited on those many occasions. Thanks to Aaron and Julie for friendship, good coffee and the Annual G' Road Xmas party.

Also, thanks to most of my many flatmates (13 in all!!!) over the last 5 years at 6 Grants Road: Julie, Catherine, Damon, Graham, Dean, Bert, Ricky, Andrew, James, Geoff, Dion, Amber and Jun.

To any I have missed please accept my apologies and know that I did appreciate your help and/or support.

Has to be said,

thanks for all the fish.... ;)

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